

Costs and benefits of carbon dioxide

THE release of carbon dioxide to the atmosphere by the burning of fossil fuels is, conceivably, the most important environmental issue in the world today. Whatever direction global energy policies take in the future, it is indisputable that carbon dioxide concentrations in the atmosphere will continue to rise. There is still uncertainty about the ultimate destination of carbon dioxide. It seems that roughly half the fossil fuel output has remained in the atmosphere, and early workers supposed that the remainder was consumed by the oceans and the biosphere. But the role of the biosphere is now a matter of hot debate. Some research has suggested that far from being a sink for carbon dioxide, the biosphere (through deforestation and changing land use) could actually be a source. Other work suggests the contrary, or that the role of the biosphere has actually changed with time. But it is inescapable that atmospheric concentrations have already climbed by 15% as a result of man's activities during this century and there seems little doubt that concentrations would be double present values around the middle of the next century if current growth rates for the use of fossil fuels (over 4% per annum) were to persist. This is unlikely, of course, given the depletion of energy resources, but at least the figure gives some sort of guide for realistic modelling.

Whatever the uncertainties about future emissions and the biosphere, there is no disagreement amongst researchers on the qualitative impact that an increase in carbon dioxide will have on climate: mean annual surface temperature will rise, and the rises will be greater at high latitudes. There is also consensus that the hydrological cycle would become more active—with precipitation and evaporation levels both rising. Beyond this there is still scope for quantitative disagreement, but a commonly quoted figure is that a doubling of atmospheric carbon dioxide would result in a world global annual mean surface temperature rise of 2 to 3 °C, with marked latitudinal asymmetry. As yet, however, no model adequately accounts for changes in the ice-covered regions of the world or in the hydrosphere (particularly ocean currents), and there is considerable room for disagreement regarding the importance of feedback effects arising from changes in cloud cover.

With so much uncertainty around, is it irresponsible

and premature to widen the debate at this stage from meteorologists and climatologists to those with interests in the consequences of climatic change—agriculturalists, glaciologists, oceanographers, economists, sociologists, political scientists and so on? Surely not, provided that sensible perspectives are maintained. A recent workshop jointly sponsored by the American Association for the Advancement of Science and the US Department of Energy has been attempting to lay transdisciplinary foundations for a federally supported research programme on the impact of increasing atmospheric carbon dioxide content and it is not too early for other nations (or more reasonably groups of nations, such as the European Economic Community) to take similar initiatives. Even if large amounts of money were not immediately forthcoming, there are still some links across the specialist boundaries which ought to be made now.

In the long run the United Nations presumably has to get in on the act, and the United Nations Environmental Programme will shortly be setting up a carbon dioxide committee. At first sight scientists might despair at the thought of yet another area in which there will be politicised conflict between industrialised nations, large-scale releasers of carbon dioxide, and the developing world, involuntary recipients of the consequences. But careful reading of what climatologists and meteorologists have to say by way of prediction makes it clear that there could be as many benefits as losses as a result of temperature and rainfall changes—and that some parts of the world may even become cooler.

There is no clear indication that the animal and plant kingdoms will as a whole prosper more or less in a changed climate. And there may be direct carbon dioxide effects, such as changes in the rates of photosynthesis and respiration, increases in the efficiency of plant water use and changes in nitrogen fixation rates. To be sure, the most widely publicised effect of a substantial global warming is the danger of the West Antarctic Ice Sheet breaking loose and melting, with highly predictable effects on sea level. But for the rest the picture is complex and by no means universally gloomy. The sooner some of the complexities are unravelled, the sooner the carbon dioxide problem can be intelligently injected into discussions of world energy strategies. □



President Carter aims to spend 'windfall' oil profits on energy research

PRESIDENT Carter last week asked members of the National Academy of Sciences to support his proposal that Congress create an Energy Security Fund, financed by a tax on the 'windfall' profits which the oil companies are expected to make following the de-control of oil prices. Such a fund could partly be used to double US investment in energy technology over the next few years.

The President's remarks were made during the course of an address to the annual meeting of the academy, the first to have been delivered to the assembled scientists since President John Kennedy spoke at a NAS meeting one month before his assassination. The Energy Security Fund, he said, which Administration officials estimate could involve collection from the oil companies of up to \$7 billion a year, would be used not only to provide relief to those least able to pay for more costly energy, but also to finance projects, including both basic and applied research, important to the country's energy future.

"By the second quarter of the 21st century, we will have learned to rely on cleaner, essentially inexhaustible sources of energy. The principal candidates include, of course, fusion and solar technologies as photovoltaics," President Carter said.

"We are preparing right now for these stages of our energy future. Our energy research and development is already larger in its programme size than those of all our allies combined. But we must do more."

The President had particularly harsh words for the oil industry, which is suggesting that the extra money it receives from increased oil prices should be used to finance its own energy development programmes, covering both exploration and research. He accused the companies of trying to 'hoodwink' the American people by passing a windfall profits tax that was in fact designed to provide the companies with loopholes through which they could increase their general revenues.

"They will try to pass this charade off on the American people as a so-called 'plough-back' provision. But it is not a 'plough back'—it is a 'plough under' and 'kick back'. And what is going to be ploughed under is the Energy Security Fund with its aid to research and to the poor," the President said.

"I ask for your support in the battle to pass an honest windfall profits tax . . . and I also call on all of you

in the scientific and engineering community to fulfil the trust of the American people by creating the new energy technologies that are so vital to the future of our country."

Although many observers in Washington feel that the President's idea of a windfall profits tax has a greater chance of succeeding in Congress than when it was first suggested last month, some have expressed doubts whether the amount of money that the Federal Government could collect in this way would in fact be as high as the President has suggested.

A report by Ralph Nader's Tax Reform Research Group has said that the proposed tax would pick up "only \$3-4 billion of the new revenues. ie, 20% rather than 50% of the increased revenues that the oil companies are expected to receive.

However the chances of Congress accepting the idea have been increased by the large profit increases which the major oil companies have been reporting for the first quarter of 1979. Texaco Inc., for example, reported an 81% increase in profits over the same period last year, and Gulf Oil Corporation a 61% increase. A spokesman for the President said that these profit increases greatly strengthened the arguments for a windfall profits tax to be used for research and other purposes.

In his speech to the academy, President Carter also asked the scientists to support his attempts to have the new Strategic Arms Limitation Treaty (SALT II), currently in the closing stages of negotiation with the Soviet Union, ratified by the US Senate.

Of all the fruits of science none is more bitter than nuclear weapons. And of all the responsibilities of nations, none is more urgent than the control of this most terrible menace to our



lives and to our civilisation," he said.

Many of the issues involved in assessing the treaty were very complex technically, and the American people would look to the scientific community to help shape an educated public debate. "Many of you devoted much effort to the debate over SALT I, and you played a major role in forming the consensus that developed to support that treaty. Today I ask for a renewal of that commitment."

The President also referred to the increasing costs of major scientific experiments—in contrast to the situation facing scientists such as Albert Einstein who required "little more than a few sharpened pencils and a quiet place to think"—and the choice that this posed between doing experiments on one's own, or in cooperation with other countries.

"We must continue to choose cooperation—for reasons that go beyond the considerable benefits of sharing costs and sharing ideas," the President said. "With our traditional friends, scientific and technological cooperation can strengthen existing bonds. With others, who may not be so friendly, it can help to bridge political, ideological and cultural division."

David Dickson

More US accidents in transporting chemicals

THERE has been a sharp rise in the numbers of accidents and deaths involving the transportation of hazardous materials such as liquefied natural gas, sodium sulphhydrate, anhydrous ammonia and chlorine, according to a report published last week in Washington by the research service of the Library of Congress. According to the report, there were 45 deaths and 1,411 injuries involving the transport of hazardous materials in 1978, compared to 32 deaths and 749 injuries in 1977 and an average of 21 deaths and 592 deaths over the previous

period. The high accident rates in 1978 had partly reflected two major incidents, the explosion of a tank-car in Waverly, Tennessee, which killed 15 persons and injured 48 others, and the de-railment of a goods wagon in Youngstown, Florida, which resulted in eight deaths and injured 158. The report says that present federal inspections may not be adequate, citing the fact that the Department of Transportation's Material Transportation Bureau has only six full-time inspectors to cover 20,000 container supply firms and 100,000 shippers. □

Critics challenge data that led to 2,4,5-T ban

THE recent decision by the US Environmental Protection Agency to issue an emergency order banning the use of the herbicide 2,4,5-T has attracted strong criticism from its manufacturers. They are contesting the EPA's decision in a Michigan district court, claiming that it is based on a "seriously flawed study" and a misreading of data by the EPA to draw conclusions to support the ban.

The ban on 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) was made when a study in the Alsea region of Oregon found a strong correlation between the use of the herbicide and a tripling in the rate of spontaneous abortions among local women. The study was conducted by Dr Eldon Savage of the Epidemiological Studies Programme of Colorado State University and commissioned by the EPA. It was the second investigation into the health effects of 2,4,5-T on residents.

The first study—"Alsea I"—was inconclusive, according to the EPA, and led to the conclusion that the claims of Alsea women that their miscarriages were due to 2,4,5-T "had not been demonstrated from the data presented." However, the spontaneous abortions did appear to follow a seasonal pattern and 10 out of the 13 cases under investigations occurred between April and September; whereas hospital records indicated that increases in spontaneous abortions normally occurred in January-March and October-December.

Alsea II was designed to eliminate any element of bias which may have occurred in the first study, and covered a much larger sample. A 1,600 square mile rural area where 2,4,5-T spraying had taken place was chosen, with another 1,000 mile rural area and an urban area as controls. Data on spontaneous abortions in the first 20 weeks of pregnancy was taken from the records of hospitals in these areas.

The study revealed that the spontaneous abortion index (ratio of abortions/live births) was higher in the study area than in the other rural area. For the months of May, June, July and August, the index for the study areas was 89.9, 130.4, 105.4 and 88.1 compared with 63.2, 46.0, 55.3 and 79.8 for the rural control. In the urban area ratios were even lower. The investigation also showed a significant correlation with the 2,4,5-T spray pattern with a lag period of two to three months. The residents, the study suggests, could have come into contact with the herbicide and its dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) contaminant in drinking water or through eating fish or other wildlife.

One critic of the Alsea II findings is Professor Nathan Mantel, a statistician at the biostatistics centre of George Washington University, Bethesda. He claims there are "fundamental statistical and logical flaws" in the report. Mantel was asked to review the report by the National Forest Products Association (2,4,5-T is in widespread use in forestry) and his comments have been forwarded to the EPA. He says the EPA were wrong to compare results for a rural study area and an urban control. His analysis revealed no statistically significant differences between the data taken from the study area and a third "control" area.

Referring to the Alsea findings of a correlation between spray times and the seasonal pattern of abortions, Mantel insists that the comparison was based on an improper statistical approach. He claimed this result was of little value anyway, since there was no statistically significant difference in the overall rates of abortion in the areas investigated—the EPA study reported little difference in the percentage of hospitalised spontaneous abortions for women aged 20-49 in the three areas.

Concern about the EPA's misreading of data centres on its use of material from Seveso in Italy. Residents of this town were exposed to dioxin in July 1976 following an accident in a reactor manufacturing trichloropheno. With dioxin known from animal studies to be teratogenic there was concern that the chemical would cause malformation to increase in the Seveso children.

The EPA says this is precisely what happened, and that there was sevenfold increase between 1976 and the first five months of 1977. While admitting that the data collected at Seveso was inadequate, and that there were "methodologic deficiencies" in the way it was analysed, the EPA nevertheless says that the evidence provides "suggestive indications of a possible teratogenic effect [by dioxin] in humans".

Many scientists insist that the EPA is wrong on this point. The EPA has been sent evidence to show that the increase in malformations at Seveso is due to better reportage of these cases and that the increase is more apparent than real. The Italian Parliamentary Commission report on Seveso reached similar conclusions and pointed out that, while many scientists believed that dioxin was potentially teratogenic in humans, the evidence to confirm this point has so far not been found. Furthermore, if dioxin had been responsible for an increase in malformations at Seveso, then there should be evidence of an increase in specific defects. However, the data does not show this pattern.

The Michigan court hearing on 2,4,5-T will have to consider these conflicting claims. A decision to uphold the EPA ban will not have serious economic repercussions on the manufacturers of 2,4,5-T through loss of sales; it will leave them open to compensation claims from women who have miscarried.

Alastair Hay

Windscale leak: Benn calls for public inquiry into safety

MR Tony Benn, UK Energy Secretary, has called for a full public inquiry by the next government into the Windscale leak which was found last month during borehole sampling near a temporary storage tank for highly active liquid waste, dissolved in nitric acid prior to concentration and final storage. At a local Labour Party meeting on Saturday, Benn expressed concern about the present leak as well as about "the continuing and unsolved leak of less active substances that has been going on for several years." Benn asked the Nuclear Installations

Inspectorate whether Windscale should be closed but was advised that this step was unnecessary.

Friends of the Earth has asked Peter Shore, UK Environment Secretary, to conduct an inquiry into other Windscale incidents which include possible leaks from the low level waste burial ground, the discovery of ^{137}Cs and ^{60}Co on surface water drains, the closure of building B204 because of radiation hazard and the discovery last year of plutonium on a half incinerated dismantled cooling tower.

The outstanding problem of the

present leak is to determine the probable rate of migration of activity from the region of the borehole. Material which has continuously leaked from the sump has progressed to a distance of 10 metres in a year. With the leak stopped, future migration depends critically on the detailed site geology. Preliminary BNFL measurements indicate that an average migration rate is about a metre a year, but according to Friends of the Earth there has never been a full geological site survey at Windscale.

Joe Schwartz

DESY makes a bid for protons in Hamburg

DESY, the West German subnuclear physics laboratory, appears to be establishing a rapprochement with CERN, Europe's international centre for the subject. It could rest on DESY building a superconducting storage ring for protons of around 280 GeV, while CERN builds LEP, a device for colliding 80 or 90 GeV electrons with positrons.

DESY would collide the protons with the electrons of its latest electron storage ring, PETRA, thus providing itself with the world's most precise instrument for probing the structure of the proton. LEP would be the most advanced tool for creating new forms of matter, such as new quarks, the intermediate vector boson, and Higgs particles.

This plan, which has yet to be formally adopted by the European high energy physics community, emerged at last week's inauguration ceremony for PETRA, held at the DESY laboratory in Hamburg. Professor Schopper, the director of DESY, told *Nature* that towards the end of this year he would be making an application to the German government to build a proton storage ring within the PETRA tunnel. The cost would be "about 1½ PETRAs"—around 150 million DM.

Earlier, the West German minister for science, Dr Volker Hauff, had made an extremely positive speech on behalf of basic science, from which it seemed clear that he would treat such an application sympathetically. "Political freedoms are inconceivable without a free science", he said unequivocally. However funds were limited (whereas research was unlimited) and choices had to be made. "We've got to limit ourselves to what we can do particularly well, where we can teach others and create top performance . . . with PETRA we do have a chance of creating this performance".

"With pride we can look forward to the development of PETRA as a centre of international research", said the minister. "More than half the researchers are guests, contributing their intellect and funds. PETRA is fundamentally enriched in this way."

And on the relationship of DESY and CERN, he said: "Very soon we will begin a dialogue with scientists to see if Germany can contribute to LEP. To have two flourishing laboratories, like CERN and DESY, is commensurate with the size of this continent. We cannot discuss the future of the one laboratory without thinking of that of the other."

This mood was reflected by Professor Jean Teillac, the President of CERN

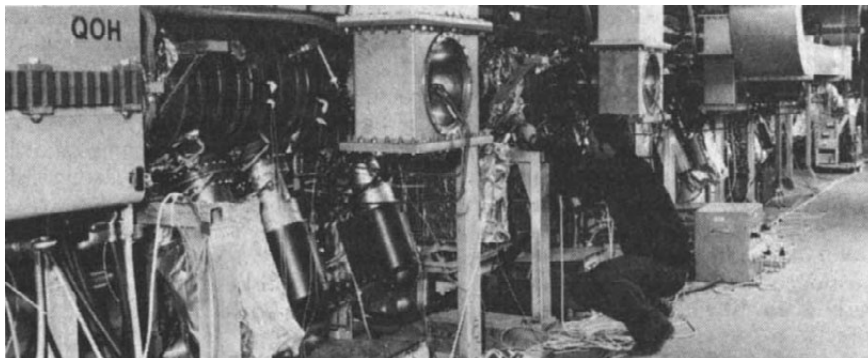
Council, the supreme body directing CERN, who said that "with CERN and PETRA Europe has a good, well-balanced programme . . . More even than in the past we must use central facilities like CERN and DESY".

Meanwhile, the new cooperative mood has yet to be translated into firm proposals by ECFA, the European Committee for Future Accelerators, which is currently deliberating on the

question of the precise energy for LEP—a delicate matter because costs increase rapidly with energy, particularly at CERN, where a big LEP would have to burrow under the Jura mountains.

"But we are not letting politics get in the way of our judgements", the chairman of ECFA, Professor Marcel Vivargent, said last week.

Robert Walgate



RF accelerator cavities at PETRA: resonances are causing beam losses

PETRA in a race to highest energy

PETRA, the world's largest electron-positron colliding beam device, is still having trouble reaching design luminosity at its highest energy—and is beginning to fear the strength of the American competition, PEP, under construction at Stanford, California.

PETRA, the brightest jewel of West German sub-nuclear physics, is hoped to make two major discoveries: the mass of the sixth quark, "top" (or "truth"), and the first indication of the masses of the intermediate vector bosons (IVBs), the particles whose exchange is believed to be responsible for the weak nuclear force. But both experiments need the highest PETRA energies, and a reasonable data-taking rate.

Last week PETRA was brought up to 26 GeV, and 10 events—the results of collisions between 13 GeV electrons and 13 GeV positrons—recorded in two detectors. But the "luminosity" (effectively, the data rate) was a factor 70 below design, producing something of the order of one event per hour. At this rate the experiment to detect the IVBs—an ambitious construction by Nobel laureate Sam Ting—would take 50 years. "Top" might be found by chance, but really requires a systematic energy scan, which would also take a long time at current rates.

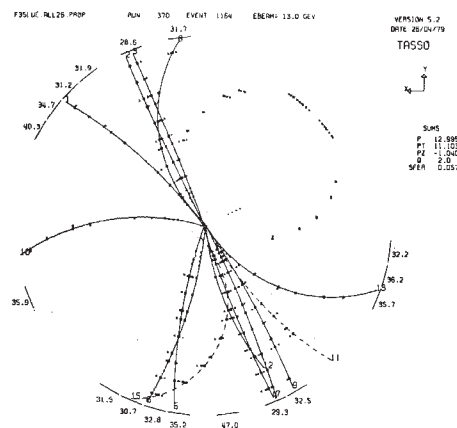
The problem appears to be due to the fact that PETRA must accelerate its own electron and positron bunches from 6.5 GeV, thus moving through a number of electromagnetic resonances in the 32 accelerating cavities. (Until now, at lower energies, PETRA

has used only four cavities.) The resonances make handling the beam a rather tricky procedure, and the beam is frequently lost.

PEP, on the other hand, which is due to come on line at the end of this year, takes injection from the Stanford linear accelerator at 17 GeV for positrons, and 24 GeV for electrons, thus scaling PETRA's 'ramping' problems at one leap.

However a glass of water may be half full or half empty, and one's view of PETRA's difficulties depends on which side of the fence one sits. On the one hand the experimenters, such as Ting's group, appear to be pessimistic; but the accelerator physicists, whose job it is to produce the beam, hand-wave hopefully. Only time will tell who is right.

Robert Walgate



PETRA's first event at 26 GeV shows jet structure

A Ukrainian line on science and education?

SCIENTIFIC research and education in the Ukrainian SSR is failing to meet the demands of the "scientific and technical revolution", according to Volodymyr V. Shcherbyts'kyy, first Secretary of the Central Committee of the Communist Party of Ukraine.

Addressing a meeting of university and college lecturers, Shcherbyts'kyy called for a "comprehensive attitude" to research, in terms which could be taken as a criticism of current Soviet policy.

Of recent years, policy has stressed the development of close contacts between academic institutions and local industry, with the scientists undertaking R & D on a contract basis as and when required. This, in Shcherbyts'kyy's opinion, is simply inadequate.

"Contracts between higher educational institutions and production are still essentially reduced to the conclusion of economic agreements with the neighbouring enterprises, and these agreements are frequently aimed at accomplishing particular, minor, tasks", he said. "A solution at the level of the entire branch practically never occurs".

What precisely Shcherbyts'kyy had in mind is not entirely clear. Presumably he would replace the present *ad hoc* trouble-shooting with a more structured programme of problem-orientated R & D, somewhat on the Polish model. Such a system, he im-

plied, would need to be organised on the regional level by the Ukrainian republic.

At present, he said, the Ukrainian Ministry of Education "has no joint plans with any [other] Ukrainian Ministry", although it is carrying out "several comprehensive plans" with the All-Union Ministry of the Chemical Industry. This lack of contact with production at the republic level, said Shcherbyts'kyy, is apparently why only about 20% of the inventions patented by scientists at higher educational institutions in Ukraine ever get applied in industrial production.

The relationship between the economies of the Union Republics and the central planning authorities is a complex one, and the relevant paragraphs of the new Soviet constitutions do little to clarify the matter.

Long ago in the 1950s Krushchev tried to over-ride the republic boundaries with a system of arbitrary economic planning regions. Although this set-up has been abandoned, at least as far as education and academic life is concerned, there has been a constant trend towards centralisation in the last two decades.

Recently, scientific publication in languages other than Russian has virtually ceased, and several promising lines of research in individual republics disappeared into main-stream anonymity. The distinctive Ukrainian

school of cybernetics of the early 1960s was probably the most notable such loss. Young graduates on leaving university are drafted wherever the economy requires—generally to the new towns of Siberia.

To date Shcherbyts'kyy has shown no signs of Ukrainian national sentiment—indeed, he was appointed in 1972 to replace Shelest, who fell from power precisely for his overly-Ukrainian attitude.

So First Secretary Shcherbyts'kyy's advocacy of strengthening academic-production contacts at a republic level seems essentially a pragmatic response to a pressing economic need, not a criticism of Soviet "nationalities policy", which to date he has resolutely supported.

On educational policy, however, he does seem prepared to make a cautious criticism. After noting "frequent cases of formalism", "unproductive use of time" and "a peculiar percentage mainia" in assessing the knowledge of students", he went on to the "participation of pupils and students in construction detachments and in agricultural operations"—officially viewed as a major factor in communist education.

Although paying official tribute to the "importance" of such activities, he nevertheless found it "necessary to emphasise that studies must be the first objective to which the student must apply his efforts". **Vera Rich**

Soviet soil erosion laboratory defends its economic record

Moscow University's laboratory of soil erosion and river bed processes is already saving the Soviet economy some 10 million roubles per annum. So said its director, Dr Roman S. Chalov, interviewed on Moscow radio last month on the tenth anniversary of the opening of the laboratory.

Appraisal of R & D in terms of pay-off and cost-effectiveness is a regular Soviet practice—although perhaps more common among policy-makers than scientists.

Dr Chalov's laboratory, however, is in a somewhat sensitive position. Last December, the Party Central Committee and the USSR Council of Ministers adopted a resolution "On additional measures to strengthen nature conservation and to improve the use of natural resource, and among the sectors singled out for special criticism, the resolution mentions soil erosion, which the planners note "is doing considerable harm to the national economy".

Dr Chalov's laboratory, being the only one in the Soviet Union specifically devoted to erosion problems, can hardly have taken this observation lightly. Chalov's anniversary interview accordingly became an apologia for the work of the laboratory. The 10 million per annum, he implied, was merely an earnest of what the laboratory hopes to do.

At present the team are largely concerned with feasibility studies. In the North Caucasus agricultural area, a study has been made of the economic efficiency of different anti-erosion measures, giving special attention to the "accelerated erosion" produced by agriculture. A further field study will be undertaken this year in the non-black soil zone of the Russian SFSR. Development of this region of relatively poor agricultural land has a special place in the current Five Year Plan. Here the team will study gully erosion—the destruction of the land by flash floods and torrents.

This extensive programme of anti-erosion work, actual and potential, has one rather surprising omission. It is entirely water-related, and has no concern with wind-erosion and desertification. This is the more surprising since Khrushchev's policy of horizon-to-horizon cultivation of the virgin lands has created a number of incipient dust-bowls.

Desert erosion, is the concern not of Moscow University, but of Leningrad. A major expedition from Leningrad University and the Main Geophysical Laboratory last summer made a detailed study of the formation and growth of dust-storms in Soviet Central Asia.

According to the teamleader, Dr Kiril Kondrat'ev, the main subject of study was the "anti-hothouse" effect, which occurs during dust-storms which extend to high altitudes, cooling the Earth's surface by reflecting solar radiation.

Vera Rich

Third World to demand new fund for scientific development

THIRD World countries are making a strong bid to have the creation of a new fund for scientific and technological development occupy a prominent position on the agenda of the United Nations Conference on Science and Technology for Development (UNCSTD), which takes place in Vienna at the end of August.

The new fund is currently referred to as the International Science and Technology Development financing system. It is among the proposals submitted to the UNCSTD secretariat last week by the Group of 77—the organisation which represents over 100 developing countries—as a possible re-drafting of a proposed programme of action to be adopted by the conference.

At present there is still considerable disagreement among members of the Group of 77 over precisely what form the fund should take and how it should operate. However, some broad principles have been agreed, such as the fact that it should operate within the United Nations system, and that it should also provide relatively stable and continuous funding, to avoid problems for scientific programmes caused by intermittent or fluctuating support.

Other proposals that have been made are that the fund should be made up of contributions based on agreed percentage of the average deficit in the trade balance in manufactured goods between the developing and developed countries; that it be financed in part through a reduction in armaments expenditure by the developed countries; and also that further funding might come from a tax paid by developed countries to compensate for the brain-drain from developing countries.

The developed countries, however, have already made it known that they will be very reluctant in Vienna to accept any proposals which will require to provide substantial increases of funds. The issue is therefore likely to be one of the most hotly discussed during the conference.

The two other main issues likely to generate debate, it emerged from last week's meeting of the conference's preparatory committee in New York, are the development of appropriate institutional mechanisms—including the rearrangement of responsibilities for science and technology within the UN system—to support any policy recommendations made by the conference, and possibly some specific proposals for ways of stimulating collaborative research on topics of particular interest to developing nations.

The developing nations are more concerned at present about financial mechanisms and about institutional arrangements—the developed nations about specific research proposals. As things stand at present it seems unlikely that any major changes will emerge directly from the conference itself (restructuring of the UN system, for example, can only be done by the General Assembly). But there are still hopes that the conference will produce guidelines representing a consensus on how each of these three items should be approached.

The main development in the conference preparations since the last meeting of the preparatory committee in New York in February has been the efforts of the Group of 77 to develop a unified position which the developing nations can present at Vienna.

A working party of the Group of 77 met in New York for the first two weeks in April to prepare a set of amendments to the preliminary draft programme of action which has now been drawn up by the secretariat under three headings: strengthening the scientific and technological capacities of developing countries; restructuring the existing patterns of international scientific and technological relations; and strengthening the role of the United Nations in science and technology and the provision of increased financial resources.

In parallel to this effort the economic commission for Latin America organised a meeting of a group of experts in Lima, Peru, in March to discuss possible financing mechanisms to provide additional support for science and technology in developing countries,

making various proposals which, although not officially endorsed by ECLA, have been partially amalgamated into the Group of 77.

At the end of last week, the Group of 77 presented to the preparatory committee its suggestion on revising the first part of the draft programme of action, that which deals with internal arrangements within countries for developing science and technology.

Many of the suggestions such as the need to establish science and technology policies as part of general development policies, and to encourage cooperation between developing and developed countries, as well as between developing countries themselves, had already been made by the secretariat itself on the basis of the suggestions contained in the various national and regional papers which have been prepared and submitted as part of the planning process for this conference.

With regard to financial resources, the group of 77 says that present funding mechanisms for enhancing national science and technology systems "are highly unsatisfactory both from the qualitative and quantitative considerations" and adds that experience has shown that "there is need for a more effective financing system within the United Nations system for strengthening the science and technology capacities of developing countries".

In general the developing countries suggest that developed countries should devote on an annual basis 0.05% of their gross national product to the solution of scientific and technological problems of developing countries, and devote at least 10% of their R & D expenditure to programmes designed to solve problems of specific interests to developing countries.

More specifically they recommend the setting up of an International Science and Technology Development Financing System to provide financial resources to supplement national science and technological financing capabilities.

Precise details of how this system would work were still under discussion by the Group of 77 at the beginning of this week. In an earlier draft of possible recommendations prepared by the working party, however, it was suggested that any new system of financing be based on a number of features, including provision that money be made available on a predictable and continuous basis, and that it should be based on a linked to international economic parameters which reflect the present asymmetries between countries in technical capacity.

Appropriate provision should be made, the working party suggested, for the financing system to involve the use of regional funds, for which the ad-



"Every time they make a bomb, we make a killing!"

ministrative responsibility would be bestowed on the developing countries themselves, while overall responsibility would rest with the UN general assembly.

Detailed discussion on these various points are likely to occupy much of the time up to the Vienna conference. Meanwhile, in an attempt to make sure that something of a more immediately practical nature emerges from UNCSTD, various countries are sup-

porting proposals that specific efforts should be made to establish projects in areas requiring greater research. Belgium, for example, submitted a resolution to the preparatory committee last week suggesting five areas for international collaborative research: solar energy, rural development technologies, tropical diseases, methods of increasing food production, and an inventory of natural resources.

David Dickson

Agricultural research centres transformed

THE dozen international agricultural research centres set up between 1959 and 1976 to conduct research into problems of tropical agriculture have had considerable influence on the national research systems of many developing countries as well as on the crops and methods used by farmers. The very changes the international centres have stimulated, however, have in turn resulted in changes being made to the mode of operation of the centres themselves so that they are no longer working to the rules laid down when the first, the International Rice Research Institute, was set up twenty years ago.

Speaking to a small gathering at the Science Policy Research Unit, University of Sussex last week, Dr Edward Clay of the university's School of African and Asian Studies said that the international centres had greatly influenced the way scientists in third world countries "perceive the research they're to carry out". In particular ever since IRRI and the International Center for the Improvement of Maize and Wheat (CIMMYT), the first two centres to be set up, achieved considerable success in breeding high-yielding dwarf varieties, most research on increasing yields for all sorts of crops in many countries has centred on finding dwarf varieties.

In India, for example, rice research has tended to focus on light-yielding semi-dwarfs for irrigated land. 67% of rice breeding is oriented towards irrigated rice, even though only 28% of the land used for rice cultivation is irrigated. And almost all the new varieties developed have been semi-dwarfs. Only 5% are suited for deep water although 40% of the land used for rice growing is under deep water.

According to Dr Clay, the international centres cannot be held entirely responsible for this mismatch of research to needs although it is highly likely that they have had some influence. In the case of rice research, for example, IRRI has mainly concentrated on irrigated rice—simply because of the local conditions around its home in the Philippines.

The centres' influence, however,

claims Dr Clay, extends beyond the individual scientist to the very agricultural policy of developing countries. "Experts were put into the international centres and were successful", he says "therefore the model of a centre was seen as a good recipe for success". More international centres were built and individual countries began to build their own single purpose centres. The Bangladesh government, for example, decided to create one specific rice research institute—"very large, costly and capital intensive with well-trained scientists". This was a new idea for an individual country—even countries with a highly organised research effort such as the US and Japan had not concentrated their research on just one topic into one place.

When IRRI and CIMMYT were first set up, they were intended to be solely research centres where Western scientists could apply certain techniques which had proved beneficial in agricultural research for temperate zone crops to tropical crops. As the influence of these first two centres spread, however, and individual developing countries began to take up their methods, they have played an increasing role in educating third world scientists and organising exchanges. With the creation of the Consultative Group for International Agricultural Research (CGIAR) "to raise funds and establish research policies" for the centres and the creation of more centres in different parts of the world, their operation has gradually become more bureaucratic.

"We now have well-defined bureaucratized institutions", according to Dr Clay. "This was not true of the original centres. In expanding and replicating the original model we've now got something quite different". There is now pressure for the centres to work much more with developing countries than was initially intended. And, according to Dr Clay, as they are essentially funded by industrialised countries this has put them into a complicated relationship between the aid-donors and their recipients.

Judy Redfearn

Pre-UNCSTD colloquium planned

PREPARATIONS for the Colloquium on "Science, Technology, and Society" to be held by the UN Committee on the Application of Science and Technology to Development (ACAST) in Vienna next August immediately before UNCSTD, were the main item on ACAST's agenda when it met in Geneva recently. At the Colloquium 200 invited representatives of the world scientific and technological community will help formulate a document for discussion at UNCSTD.

During the first week of the ACAST meeting it became apparent that the Colloquium is seen only in terms of certain items of the agenda of UNCSTD. For whatever reasons, the members of ACAST seem to have forgotten their hitherto jealously guarded role as an independent advisory committee, and limited themselves mainly to points they had been asked to consider by UNCSTD Secretary-General, José da Costa.

However, da Costa himself possibly helped to harden ACAST's attitude. His personal intervention on a flying visit, during which he did not even stop to answer many of the questions he inspired, appeared to reflect considerable pessimism as to the outcome of his own Conference. Seeing less than a fifty-fifty chance of any concrete results, he feared it would be only a "conference verbale", within which ideas on, amongst other things, new forms of cooperation between North and South, as well as within the South might at best appear in the form of recommendations.

Da Costa's statement was also noteworthy for a sudden and highly emotional attack on the way in which his conference is being treated by the media, in particular by the "Lund Letter", published by an internationally-oriented group in Sweden, and which he singled out as "nordique et paranoïaque".

A second factor which further enlivened the ACAST proceedings was what looked remarkably like an attempt on the part of UNESCO, through their representatives at the meeting, to take over the whole Colloquium, albeit still disguised as an ACAST-sponsored meeting. Whether this was because of their frustration at the admittedly somewhat tardy and, to some observers, impractical way in which the Colloquium was being planned and organised, is not clear.

However, here the members of ACAST closed their ranks and refused to be stampeded by UNESCO's probably justified calls for urgency.

Peter Collins

news in brief

UN urged to take lead in studying effects of CO₂: The United Nations Environment Programme can and should take the lead in assessing the global impact of an increase in carbon dioxide on the environment and health and society generally, Mrs Barbara Blum, deputy administrator of the US Environmental Protection Agency, told UNEP's governing council in Nairobi last week. She also called for an international meeting of experts to accelerate and coordinate action to improve management of the world's forests. "Tropical forests are the world's richest generic reservoir, a potential source of useful plants and drugs, a modulator of climate, a shield against desertification and soil loss, and a renewable timber-bank", she said. "It is time to highlight this key problem for decision-makers at the highest level of government."

Recent experience in the US, which was now having to clean up the consequences of earlier indiscriminate dumping of toxic wastes, showed that the land was not an infinite sink or sponge which could be used as a dumping ground for residues of industrial activity.

Russian invitation: France has been asked to supply a cosmonaut for a space flight with a Soviet crew. This proposal was made by Mr Brezhnev to President Giscard d'Estaing, during the French leader's visit to Moscow last week. According to Tass, Giscard "responded positively".

TUC gets in on microprocessors: Two weeks after the UK Science Research Council jumped into the technology of microprocessors with proposals for a major research and training programme (19 April), the Trades Union Congress has urged full cooperation between government, management and the unions to avoid the loss of "hundreds of thousands of jobs". Mr Len Murray, TUC general secretary, notes in a report that "in the absence of intervention and planning, the benefits of microelectronics will certainly be unequally distributed". The TUC calls for a 35-hour week, a reduction of systematic overtime, longer holidays and sabbaticals and trade union education in microprocessor technology. But Professor Kristen Nygaard of the Institute of Informatics of the University of Oslo says that the key issue is one of workplace control. He cites the introduction of "data" shop stewards in Norway as a more appropriate demand for trade unions to make. The shop stewards serve on small shop floor committees of four to six persons and they are not permitted to become programmers. The stewards are also required to attend the annual data processing course given by the Norwegian TUC that has been held since 1971. *Employment and Technology. TUC, Great Russell St., London WC1. 40p.*

Cornell ring starts storing electrons: The first circulating beam of electrons has been stored at Cornell University's new Electron Storage Ring, a facility which, when fully operational later this year, will enable stored beams of electrons and positrons to collide at two points in their orbit. The storage ring is mounted in the same tunnel that houses the Cornell electron synchrotron, which will provide the high energy electrons and positrons for injection into the storage ring. Conversion of the synchrotron into a colliding beam facility has been under way since September 1977, and has been funded at an estimated cost of \$20.7 million by the National Science Foundation.

Head of FDA resigns: Dr Donald Kennedy has resigned as director of the US Food and Drug Administration, an agency of the Department of Health, Education and

Welfare, to return to Stanford University in California where he is to become provost and vice-president for academic affairs. Before joining the FDA in March 1977, Dr Kennedy was professor and head of the human biology programme at Stanford. For a time he was also presidential science adviser, acting as one of two senior consultants to the Office of Science and Technology Policy. Announcing his decision to return to academic life, Dr Kennedy said he had intended to stay at FDA through a full academic term, "but the position at Stanford will not wait". He leaves the FDA on June 30—one day before a tough new ethics law restricting the contacts that ex-government employees can have with their previous agencies comes into effect—and starts work at Stanford on August 1.

In his two years at the FDA, Dr Kennedy has been at the centre of a number of controversial issues. He has strongly supported attempts to ban saccharin as a potential carcinogen, and has strongly opposed moves to legalise the medical use of the claimed anti-cancer agent laetile. Last year he was also behind unsuccessful attempts by the administration to overhaul FDA's drug-regulation system.

New analysis traces consequences of world resource depletion: Worldwatch Institute, a Washington-based non-profit research organisation, has released a study on the economic effects of population growth, which finds that significant per capita declines are occurring in four major resource areas: fisheries, forests, croplands and grasslands. Over-fishing has become the rule rather than the exception. In forestry, demand has exceeded the sustainable yield of the world's forests producing higher costs for lumber and a consequent shortage of housing. Grassland management is characterised by overgrazing on every continent and pressure on beef, milk, wool and leather production. Crops are also strained, and cereal production, which increased 30% from 1950 to 1971, has levelled off.

The study points out that when biology starts to fail, oil tends to be substituted, thus placing a drain on energy reserves. Synthetic fibres now account for a third of the market and synthetic rubber two-thirds. Cardboard and wood are replaced by plastics. In addition, agriculture depletion increases the demand for chemical fertilisers and mechanised agriculture to maintain production. Worldwatch says; "The oil safety valve is starting to stick." The study is critical of supply and demand solutions and sees a need for government intervention in population policy and resource management and increased research into the capacity of the world's biological systems.—*Resource Trends and Population Policy: A Time for Reassessment. Worldwatch Paper 29.*

Sino-Bangla technical cooperation: China and Bangladesh have signed an agreement for the mutual exchange of scientists and technicians to study agricultural production. China will organise study visits for Bangladesh experts in the freshwater fish farming, paddy rice culture, small irrigation projects and agricultural machinery manufacture. Also included will be the processing of green tea and Chinese medicinal herbs and visits to study the use of natural gas for producing chemical fertiliser. Bangladesh will offer study visits on jute manufacture, black tea growing and cultivation of mango saplings. About 50 Bangladeshis will visit China in the first group which will leave in the next two months. Bangladesh has been aligned towards Western technological development but the Chinese agreement may open up opportunities for the transfer of traditional technologies based on local resources.
—from M. Kabir in Dacca.

Multi-mirror telescope confounds the sceptics

David Dickson reports on a telescope with a radical new design which is to be officially inaugurated in New Mexico next week

FROM the main road it looks like a huge, gift-wrapped Christmas present, perched precariously on the summit of the 8,500-ft Mount Hopkins, 25 miles south of Tucson, Arizona. Only as you cover the last, steep stretch of the approach road and begin to glimpse the apparatus behind the rolled-back doors, does the building's function as an observatory become apparent—despite the absence of the conventional split dome.



Even then, the surprises are not over. Rather than the long, carefully balanced cylindrical telescope that one expects, there is a squat, almost cube-shaped construction with six identical mirrors hidden in a complex of steel girders, the whole apparatus supported by a massive steel yoke.

The Multi-Mirror Telescope, has been built jointly by the Smithsonian Institution and the University of Arizona, and is to be officially inaugurated next week. Attending the dedication ceremony may well be some of those who were initially sceptical about the feasibility of combining six separate reflecting mirrors in a single telescope, a step which the designers claim to be the first major departure from conventional optical telescope construction in more than a century.

"It is a radical design, and it met with an enormous amount of scepticism in the beginning. Even those involved in the project found they had to overcome some of their own biases and prejudices", says Professor William F. Hoffmann, of the University of Arizona's department of astronomy. "Many doubted whether it was practical to trade off the cost of a single, large primary mirror against the complexity of the control and alignment systems needed to operate a number of smaller mirrors simultaneously. And even if the trade-off proved to be worth it, there were doubts about whether the control system could be made to work adequately—and to run routinely."

Some of these reservations—and in particular the last one—have yet to be answered. But preliminary viewings have already been successful, even

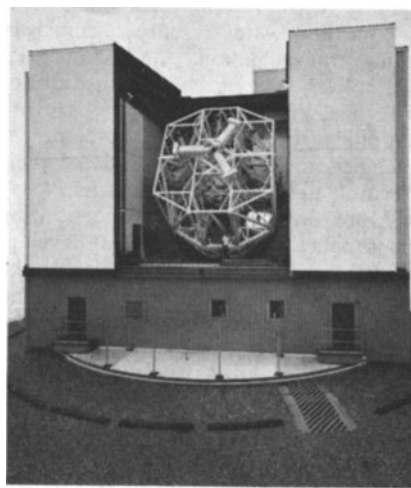
though the telescope has to be operated manually until the computerised control system is linked up. Professor Hoffmann says he does not foresee any major problems.

There are three radical innovations in the MMT design, each of which is being closely followed elsewhere as suggestion new directions in telescope construction. The MMT is really a combination of six parallel Cassagrain telescopes, each with its own 1.8-metre primary mirror and its own secondary mirror, with the separate images focused to a single point. This results in a light collecting capability equivalent to a 4.5-metre telescope—still behind the 5-metre telescope on Mount Palomar and the Russian 6-metre telescope, but its compact design means that, at \$7.5 million (in 1975 prices), the MMT cost less than a third of a conventional design.

Laser beams align the mirrors, using the MMT's guide-alignment telescope as a collimator for a laser-generated point source. The beam is split into six components, each used to keep a check on one of the mirrors and control its fine-adjustment mechanisms. "The problem is comparable to getting six headstrong ballerinas to dance as if they were one," says one of the project directors.

MMT may well serve as a prototype for a new generation of optical telescopes, avoiding the increasingly prohibitive costs of large primary mirrors. Already a multi-mirror design is being considered as one option for a 10-metre telescope being planned by the University of California. Scientists at the neighbouring Kitt Peak National Observatory are exploring a similar approach to a 25-metre instrument.

The second design innovation is the



Complex of steel girders hides the mirrors

mounting. Most conventional telescopes use an equatorial mount, meaning that movement about only one axis is required to compensate for the rotation of the Earth. The MMT, in contrast, uses an alt-azimuth mount, the horizontal axis being provided by a large steel yoke which itself turns on a vertical axis. Keeping track of an object during viewing means rotating the telescope about both axes simultaneously, a process which is controlled by a special mini-computer.

Both the compact design of the telescope, and the alt-azimuth mounting, led to the concept of a rectangular housing. "Any dome is, in its own geometry, azimuthal, but with an equatorially-mounted telescope you need the extra swing-space within the dome," says Professor Hoffmann. "With the alt-azimuth mount, motion is either up and down or circular, so saving space inside the dome is no longer necessary. A rectangular space then became much more convenient than a spherical one."

Professor Hoffmann says that although when first suggested the fork mounting and the rectangular building were considerable innovations, it turned out to be so practical that he thinks "people will soon find that the hemispherical dome and the equatorial mount are no longer attractive". Scientists from the Australian National University are already contemplating using this approach for a new telescope on Mount Stromlo, and others are expected to follow.

When it becomes fully operational, the MMT, which will be able to operate in both visible and infra-red parts of the spectrum, will have a number of uses. It will be able to carry out infra-red photometry that could help scientists identify materials on the surface of Pluto and the satellites of Uranus and Neptune, and also provide detailed measurements of highly red-shifted optical spectral lines from distant quasi-stellar objects.

Another major application will be in spectroscopy. By projecting the images from the six main mirrors along a line, rather than on to a single spot, the MMT will be able to compensate for the light lost when the single image from a conventional telescope is focused on a single slit.

With other ground-based facilities—particularly those at Kitt Peak—increasingly in demand, there will be no shortage of users for the MMT. "They are already beating a path to our door," says Professor Hoffmann. Initial users will be those from the Smithsonian and the University of Arizona. Subsequently, applications for experiments will be open to all comers. □

'North' meets 'South'—without the bickering



Roger Revelle reports on hopes for a new era of scientific and technical cooperation in the Third World

FOR a variety of reasons, including the probable makeup of the national delegations, the United Nations Conference on Science and Technology for Development (UNCSTD) to be held in Vienna in August, will be largely political and diplomatic.

So to provide a forum for scientists and engineers to express their ideas for UNCSTD, the International Council of Scientific Unions (ICSU) enlisted the cooperation of other international professional organisations (including those of the social scientists, humanists, physicians and engineers, and such quasi-scientific groups as the Club of Rome and Pugwash), in sponsoring an International Symposium on Science and Technology for Development, which was held in Singapore during the week of 22 January under the chairmanship of Thomas Malone, Treasurer of ICSU and Foreign Secretary of the National Academy of Sciences. Some 120 natural and social scientists and engineers from forty countries participated, besides observers from the United Nations and its specialised agencies, and from national technical assistance organisations. More than half the participants came from the developing countries of Africa, Asia, and Latin America, while the remainder represented both the capitalist and the 'centrally planned' industrialised countries. Notably absent were representatives of the People's Republic of China—which has had difficulties with ICSU over Taiwan—and the oil-rich Arab countries, though scientists from Egypt, Sudan and Morocco actively participated.



Thomas Malone

Some of the more important conclusions of the symposium were:

- The application of science and technology for development depends very largely on the existence of effective scientific and technical institutions within each developing country. Through these, each country can improve its indigenous technology,

make rational choices among foreign technologies, absorb chosen technologies, and create new ones appropriate for its special circumstances. These institutions will need to conduct some basic and applied research, as well as technological development, in order to retain a competent staff and to stay aware of scientific advances throughout the world that may eventually become useful to their country.

- To build up their scientific and technical capabilities and their industries, the developing countries require a greatly increased corps of qualified technicians and other skilled workers. A large number of technical schools and junior colleges should thus be established in the Third World, and should be open to both sexes to expand employment opportunities for women. Apprenticeship training for LDC technicians in the industries of the developed countries and in multinational corporations in their own countries should be strongly encouraged, while school curricula in science and technology need expansion and modernisation, with emphasis on experimental work and manipulation of equipment.

- The establishment of regional research and development institutions (analogous to the present international agricultural research centres) should be considered, covering a wide range of scientific and technical fields, including forestry, fisheries, management of land and water resources, construction, transportation, and industrial technology. These regional institutions will need to work closely with the national research and development agencies, to ensure the results of their research are put to practical use.

- More donor agencies concentrating on support of research and development in and for the Third World, like the International Development and Research Centre (IDRC) in Canada, the Netherlands University Foundation for International Cooperation (NUFIC), the Swedish Agency for Research Cooperation with Developing Countries (SAREC), and the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) should be established

in the industrialised countries. Existing agencies should be encouraged to co-operate more closely with each other in exchanging information and ideas and in creating interacting programmes. The outstanding work of the International Foundation for Science and the United Nations University, in supporting applied research by LDC scientists within their own countries, deserves greater financial backing by the developed countries.

- Physical and biological scientists and engineers in the developed countries need to be more aware of the developing countries' underlying problems, whose solution demands both basic and applied research; they should be encouraged to undertake such research in cooperation with scientists from the Third World. For this purpose, the international scientific organisations need increased participation by scientists of the developing world, and improvements in their analysis and communication in areas related to developing country problems. UNESCO and other specialised UN agencies could help the non-governmental organisations play a more effective role. International 'workshops' focused on specific problems, in which scientists from developed and developing countries jointly formulate research and development programmes, can serve as a catalyst for cooperative efforts.

One distinguishing aspect of the symposium was the range of talent, experience and knowledge among the participants; another was the absence of the harsh confrontations between the "North" and the "South" that have frustrated useful discussion in many recent UN-sponsored conferences. Instead, it seemed clear at Singapore that a new international scientific and technical order is an essential prerequisite for the new international economic order so earnestly desired by the countries of the Third World.

The symposium considered ways in which science and technology could be used to alleviate some of the most critical developing country problems. The real incomes of the poorest classes need to be increased, by reducing unemployment and increasing productivity by means of appropriate rural and urban industries, which can be created through applied research, or by adaptation of existing technology to utilise available resources. But technical and managerial skills in the rural and urban labour forces must also be developed, and the tools of the social scientist must be used as a guide to choices of industrialised technology.

These industries will require considerable increases in energy supplies, particularly in rural areas, and hence the development of new sources of energy and new means for energy conservation. The energy problem is also related to the need to increase and diversify agricultural production, to develop forest resources and to improve transportation.

Natural and social scientists must also work together to bring about the social and economic improvements in health that will help to slow down population growth and ultimately stabilise the size of populations.

Throughout the symposium, special emphasis was given to the role of women in the development process, in particular the need for greatly expanding women's opportunities for employ-

ment and education, and for relieving the unallayed drudgery that characterises traditional 'women's work' in rural societies. The unfavourable socio-economic environment for women would be improved if they could participate more fully in development—the less-developed countries cannot reap the full benefits of technology until this vital human resource has been mobilised. Conversely, the multiple impacts of technology on the welfare of women in changing societies must be evaluated.

Industrialists in both developed and less-developed countries will inevitably have a central role in the application of science and technology to development. To serve their own long-range interests, industries operating in each developing country should be en-

couraged to choose technologies and a scale of operations that are most appropriate for the local factors of production and lead to greater equity within the country, as well as improving the human and natural-resource base.

The participants were unanimous in agreeing that the Singapore symposium should not be an isolated event. A continuing steering committee was established which has begun to explore those problem areas that could be most effectively approached by cooperation among scientists and technologists from the rich and the poor countries. □

Roger Revell has been director of Scripps Institution of Oceanography and more recently director of the Harvard Center for Population Studies.

UNCSTD: gloom over growing gulf between politicians and scientists

"THE gap between the 'experts' and the policy-makers, between those who study and work with science and technology, and those who make development decisions—that gap seems to be growing ever larger as UNCSTD draws near", laments the latest issue of the *Lund Letter*, a Swedish commentary on preparations for the conference.

And a recent Swedish Royal Academy of Sciences meeting has emphasised the point. Called to inform and stir up interest among scientists who may not have been involved in the official preparations for the conference, the day-long discussions left a pessimistic impression of what the official UNCSTD session can hope to achieve.

The non-governmental organisations, however, offer more hope. They came up with some good ideas at their meeting in Singapore in January (see above), and the academy meeting pointed to several areas where Swedish research could be useful to the Third World. The non-governmental organisations seem certain to make a hefty intellectual and psychological contribution.

One suggestion for a new form of cooperation came from Dr Stephan Schwartz, the head librarian at the Royal Institute of Technology, who wanted to improve the flow of scientific and technical information to developing countries. From his own survey of the age distribution of references in scientific journals, it is apparent that in a good journal (*Nature* was his example), half of the references are less than two years old. In a journal used in even a relatively advanced developing country like Portugal, half the references are seven to 10 years old.

This means that the Third World is not getting the most up-to-date information, and is therefore not able to tackle its problems in the most up-to-

date ways. This situation could be improved by establishing link-ups between libraries in developing countries and Western institutes which could do computer searches of the specialised literature needed to cope with the countries' particular problems. Photocopies of most important material could then be supplied to the developing countries.

Dr Schwartz pointed out, however, that the information such a system could supply would be useless unless it could be assimilated by the local science and technology system. He thus echoed one of the meeting's recurrent themes: that development of one sector in a country is only possible if it is backed up by development in all the other sectors. It is these gaps that are hardest to close, because they are the result of development and depend on all other gaps closing first.

The gaps in the politicians' outlooks are so wide as to make them incompatible. In agriculture alone, Asian and African demands are totally different from what the Europeans want to offer.

As Mr Thomas Rosswall from SCOPE (the Scientific Committee on the Problems of the Environment, set up by the International Council of Scientific Unions) pointed out, the European Community (EC) paper presented to UNCSTD's Third Preparatory Committee in January maintains that it is possible, with adequate water and fertiliser supplies, to triple and quadruple yields from traditional varieties of seeds. This is irrelevant to the Third World, where neither adequate water supplies nor fertilisers are available.

In talking about the advantages of fertilisers, the EC paper takes no heed of the fact that widespread use of fertilisers is only possible where their cost, relative to the costs of other agricultural inputs, is low, which is certainly not the case in developing

countries. Again, the EC paper, recounting the European experience, sees mechanisation as a good way of achieving economies of labour. But the Asian and African papers specifically say that they want labour-intensive agricultural systems.

On a more practical note, Dr Bo Hall, of the Department of Agriculture's Commission on Natural Resources, pointed to research on the production of pig iron on a small scale but at prices competitive with the iron produced from big blast furnaces. Small-scale methods are needed for the exploitation of small domestic markets, in contrast to the large-scale technologies developed by the multinationals for developing large ore bodies.

Dr Malin Falkenmark, of the Natural Sciences Research Council's Committee for Hydrology, said that research on groundwater in hard rock is a Swedish speciality and could be useful in India and West Africa especially, where rock groundwater is an important source of water. Groundwater replenishment techniques—techniques for artificially recharging groundwater supplies—were originally developed in Sweden and could probably be used widely in arid and semi-arid areas.

There are vast regions where Swedish work on erosion and sedimentation could also be applied. These processes are not always thought of in relation to river basin development, although many reservoirs rapidly fill up with sand if erosion is not stopped on lands further upstream. Finally, the Nordic countries could pool their work on water assessment—determining the available water resources in an area to avoid over-exploitation—and help developing countries to make water assessments of their own.

Wendy Barnaby

correspondence

Jobs: the need to reach an ideal steady state

SIR,—The problem of short-term contract workers is a serious one but it is also very complex and one should beware of facile solutions which can have even more disastrous long term repercussions.

To illustrate the problem one has to look at the ideal steady state. In the biomedical sciences we estimate that there are about 5,000 tenured academic and research posts in United Kingdom Universities and research institutes. There is probably about the same number in the physical sciences. Since each tenured post is occupied for about 35 years the average turnover rate, assuming no wastage, should be about 2.8% per annum. With a uniform age distribution, we could expect 130 vacancies every year in the biomedical sciences simply for renewal. It would mean that we could have a total of 400 people in three year postdoctoral fellowships with excellent chances of their being placed in tenured posts at completion.

The problem is that we are very far from this situation. In 1976, only 20% of the science posts in British universities had holders aged 50 and above, which means that for the next 15 years the retirement rate is going to be about 1% and we can only expect positions to appear about $\frac{1}{3}$ of that expected from the ideal steady state. Thus the biomedical sciences will only have about 30 to 40 vacancies a year which not only accords more with what we know to be the case, but also tells us that this will be with us to very near the end of the century. The reasons are obvious; this generation is now paying the price for the rapid change in the rate of expansion in the late fifties and sixties; the people they are replacing were appointed before that when the system was much smaller. It is also easy to see that if the step at the below 40 age group is followed by a trough, the long term consequences could be disastrous.

The only solution is to aim to get to the ideal steady state position now. Tenured posts must be offered in universities at the average turnover rate, but in order to do this the system will have to undergo an absolute expansion. Naturally this bulge should not enter into the calculation of the turnover rate, but should be seen only as a borrowing against future vacancies. It seems to me that, in the main, this has to be done largely by government intervention as part of a deliberately planned policy. Nevertheless, there are contributions which might be made by private foundations and possibly by research councils. The best they could do would be to create senior research positions in universities for people already holding university posts. These would all be *ad hominem* appointments so that the subject areas and the candidates are selected by these organisations, and the posts are not simply given away with no control. However, the position vacated by such an appointment can now become available to the university for a new entrant. In essence, in this scheme, the outside organisation is only temporarily

committing itself to a university for the length of tenure of the post, perhaps 15 years; for new tenured entrants, the commitment is for 40 years and only the universities can do that.

From this analysis it can be seen that temporary posts do not help, except as offering some small buffering capacity. They can only be created and used effectively against future vacancies, and unless accompanied by other actions they can only worsen the situation. A prolonged postdoctoral period can be seen as a mechanism which gives the occupants several additional opportunities to apply for permanent posts as they arise annually. This does not help as long as the input further back in the system provides excessive competition, and the difficulties are further compounded by the universities choosing younger candidates for lectureships because they are cheaper than the man with several years experience. We have also to tackle the question of the number of people doing PhD degrees, and this raises a whole new set of problems. What is a PhD degree for? If it is seen as the first step towards a research or academic career, and this is the expectation of people doing it, then we are producing too many, and the numbers should be cut. Universities would strongly oppose that step, because PhD students provide a cheap source of research labour in many university science departments. We have to look at postgraduate education and training as a matter of urgency; it is clearly impossible and undesirable for every university to have a graduate school in every subject, since that puts demands on resources that cannot be met.

The question of the short-term workers is only the tip of the iceberg and the central problems are deepseated and long term. In some way, everybody involved needs to be got together to find a national solution; and unless this is done soon and in a concerted manner I, for one, am very nervous about the long term future for British science and higher education.

SYDNEY BRENNER

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Little danger from radon

SIR,—Recently, *Nature* reported (30 November 1978, page 431) the work of Berkeley scientists who have expressed concern that reduced ventilation rates may increase the hazard from the inhalation of the short-lived daughters of ^{222}Rn . This problem has been under consideration by the National Radiological Protection Board in recent years and we think that the conclusions reached by the workers at Berkeley should be approached with caution.

They based their case on extrapolation from uranium miners' medical data. For uranium miners, W. Jacobi (Proceedings of NEA Specialist Meeting, Elliot Lake, Canada, OECD, Paris pp. 33–38; 1977) has estimated 200 excess lung cancer deaths among a population with a cumulative exposure of 10^6 Working Level Months. (The Working Level Month

Table 1 Lung cancer incidence predicted due to environmental radon daughter exposure

Winter ventilation rate h^{-1}	Mean population exposure WLM y^{-1}	Lung cancer cases predicted per 10^6 population per year
0.8	0.15	30
0.5	0.22	44
0.4	0.28	56
0.3	0.38	76
0.2	0.58	116
0.1	1.15	230

(WLM) is 170 hours exposure at 1 Working Level (WL); the Working Level is a measure of the concentration of the potential alpha energy in air from any combination of the short-lived daughters of radon. $1 \text{ WL} = 1.3 \times 10^6 \text{ MeV m}^{-3}$). A recent survey (Cliff, K. D., *Phys. Med. Biol.* **23**, 696–711; 1978) estimated that the average population exposure rate domestically to radon daughters for Great Britain is about 0.15 WLM per year.

From the above figures it is possible to deduce, for the female population of Great Britain (assuming a 20 year latency period) that inhalation of radon daughters has caused more bronchus and lung cancer deaths up to the age of 40 than actually occur from every single cause, according to mortality statistics. This result shows the danger of applying a risk estimate derived from a special group to other groups of the population. Even if allowance were made for more rapid room ventilation in the days of open fires, we would still deduce that lung cancer incidence among women under 40 was entirely attributable to radon, and that smoking made little contribution—an untenable conclusion.

Is a WLM to a working miner the same, from a comparative dosimetry point of view, as that to a member of the general population? Studies show that, to within a factor 2, there is no difference in the deposition of radon daughters in the lung. In the absence of any other data, apart from that for miners, on which to base a risk estimate for the general population we may use Jacobi's figure to consider the implications of a general reduction in ventilation rates during winter. We assume a mean ventilation rate in summer (5 months) of two room changes per hour and that energy conservation efforts will not alter this rate. Table 1 shows the predicted incidence of lung cancer in the population (male and female) of Great Britain as the mean winter (7 months) ventilation rate is reduced. The current total lung cancer incidence in Great Britain is about 650 per 10^6 population per year.

It cannot be stressed too strongly that such calculations as given in Table 1 should be regarded as speculative and represent an absolute upper limit to possible lung cancer incidence attributable to environmental radon daughter concentrations.

K. D. CLIFF
B. L. DAVIES
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news and views

Clinical relevance of opiate receptor and opioid peptide research

from Solomon H. Snyder

OPIATE receptors¹ and the enkephalins, their endogenous peptide ligands², have advanced the understanding of numerous brain functions and provided several potential clinical applications.

The localisation of opiate receptors as revealed in the elegant autoradiographic studies of M. J. Kuhar³ has clarified the neuronal sites at which these drugs exert their pharmacological effects. Opiate overdose causes death by depressing respiration. High densities of opiate receptors in the nucleus of the solitary tract, a respiratory reflex centre, suggest likely sites of this activity. Opiates constrict the pupils, accounting for the 'pinpoint' pupils of heroin addicts, an effect apparently attributable to opiate receptors in the pretectal nuclei. Opiate receptor concentrations within the limbic system may mediate euphoric effects. If opiate receptors vary in drug specificity throughout the brain, as suggested by pharmacological data⁴, novel drugs acting selectively on individual receptor populations might afford selective therapeutic advantage.

Opiate addiction

The opiate receptors which are highly concentrated in the locus coeruleus may have direct relevance for treating opiate addicts, especially their withdrawal symptoms. Though the locus coeruleus contains only 1,400 neuronal cells, all contain noradrenaline and their axons project ubiquitously, providing the major adrenergic innervation of the central nervous system. Micro-injections of opiates selectively inhibit firing of locus coeruleus neurones but fail to alter the activity of closely adjacent cell groups which do not possess opiate receptors⁵. Besides opiate receptors, locus coeruleus cells also possess α_2 -adrenergic autoreceptors, whose stimulation by noradrenaline or clonidine, which mimics noradrenaline at α_2 -receptors slows their firing⁶. Mor-

phine tolerance and physical dependence in rats is accompanied by tolerance of the locus coeruleus to the slowing effects of opiates but not of clonidine⁶. Opiate withdrawal in these rats elicits extremely rapid firing of locus coeruleus neurones.

Drugs which block the α_2 -adrenergic receptors, such as piperoxane and yohimbine, accelerate the firing of locus coeruleus neurones and produce subjective effects of anxiety and agitation as well as hypertension in humans. As these symptoms resemble opiate withdrawal, some abstinence symptoms might result from enhanced locus coeruleus activity. If this were the case, drugs slowing locus coeruleus neuronal firing might alleviate opiate withdrawal symptoms. Gold *et al.*⁷ observed a dramatic alleviation of abstinence symptoms in human methadone addicts treated with clonidine. Thus, clonidine or related agents may prove useful treatments for opiate withdrawal. Conceivably, maintenance therapy with such drugs would ease the craving of addicts for heroin.

Developing new analgesics

Simple test tube assays to measure opiate receptor binding have greatly facilitated the screening of potential new opiate-related drugs. More important, the discovery that low concentrations of sodium ion selectively differentiate the actions of agonists, antagonists and mixed agonist-antagonists at opiate receptors may facilitate development of potentially less addictive opiates⁸. Sodium 'weakens' the binding of pure agonists to opiate receptors by 15–100-fold, while not influencing the binding of pure antagonists at all. Drugs with both agonist and antagonist activity are affected in an intermediate fashion. Before biochemical labelling of the opiate receptor these mixed agonist-antagonist analgesics, which have reduced addictive potential, were difficult to detect by tests in intact animals. Opiate receptor screening has simplified their identification. Until recently, pentazocine (Talwin) was the only mixed agonist-antagonist opiate available

commercially. Though less addictive than conventional opiate agonists, pentazocine does not relieve severe pain as well as does morphine. At therapeutic doses pentazocine sometimes elicits anxiety, depersonalisation and psychotomimetic effects. A 'second generation' of mixed agonist-antagonist opiates are now appearing on the market. Butorphanol (Stadol), from the same benzomorphan class as pentazocine, is more potent and might conceivably have fewer psychotomimetic effects. Nalbuphine and buprenorphine are very promising mixed agonist-antagonists which may have even greater potential, but are not yet widely enough used to judge their liability to cause addiction.

Enkephalin analogues

Numerous pharmaceutical concerns have developed enkephalin analogues for possible therapeutic uses, hoping that derivatives of such a natural substance might be non-addicting. The amino acid sequence of Met-enkephalin is tyrosine - glycine - glycine - phenylalanine-methionine, while Leu-enkephalin differs from Met-enkephalin simply by the substitution of a carboxyl-terminal leucine for methionine. Replacement of the glycine at position 2 by D-alanine greatly reduces the proteolytic susceptibility of enkephalin. Other modifications increase receptor affinity, facilitate penetration of the blood-brain barrier, and enhance absorption from the gastrointestinal tract. The Sandoz Company reported a pentapeptide FK-33824 which differs from Met-enkephalin only in the replacement of glycine-2 with D-alanine, the N-methylation of phenylalanine and alteration of the methionine by oxidising its sulphur to a sulfoxide and conversion of the carboxyl to a carbonyl⁹. This drug is 30,000 times more potent than enkephalin when injected into the brain, is an active analgesic when administered parenterally and orally and has already been evaluated in humans¹⁰. The facial flushing produced by FK-33824 resembles the naloxone-reversible facial flushing in

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some diabetics given the anti-diabetic drug chlorpropamide followed by alcohol, implicating enkephalin in the pathophysiology of certain types of diabetes¹¹. In some animal tests, FK-33824 elicits physical dependence, but is less 'addictive' relative to its analgesic activity than are conventional opiate agonists (D. Romer, personal communication). A possible successor to FK-33824 has already been developed simply by adding an N-methyl group to the tyrosine¹². The resultant methyl-FK-33824 is five times more potent than FK-33824 by subcutaneous injection and orally. Its oral form is equipotent with morphine.

The Lilly Pharmaceutical Company has developed an enkephalin analogue differing from 2-D-alanine-Met-enkephalin simply by methylation of methionine's amino nitrogen and conversion of methionine to an amide. This agent, LY-127623 seems to have similar potency to FK-33824 (refs 13, 14) when administered parenterally but is less active when given by mouth.

LY-127623 may be less addictive. Withdrawal symptoms in morphine-addicted rats are much less severe with LY-127623 than with other conventional opiate agonists or mixed agonist-antagonists examined. Also, at equi-analgesic doses LY-127623 produces less respiratory depression than morphine. In numerous pharmacological tests LY-127623 and FK-33824 behave

like pure opiate agonists. Thus these enkephalin analogues may be unique as agents outside the mixed agonist-antagonist group with reduced propensity to cause addiction. The absence of direct comparisons between LY-127623, FK-33824 and other enkephalin analogues precludes conclusions about their comparative benefits.

Drugs which inhibit enzymes that inactivate endogenous substances are often powerful therapeutic agents—for example, acetylcholinesterase and monoamine oxidase inhibitors. Enkephalin can be degraded by a wide range of nonspecific peptidases. Recently Malfroy *et al.*¹⁵ identified a specific 'enkephalinase' in brain membranes. This enzyme has a much greater affinity for enkephalin than for most other peptides. Its regional distribution throughout the brain resembles that of the opiate receptor. Moreover, in rats chronically treated with morphine, enkephalinase activity increases substantially, while other peptidases show no change. Drugs which facilitate enkephalin action by blocking this enzyme might have unique applications extending beyond the opiate field. Enkephalinase seems closely similar to the angiotensin-converting enzyme which transforms the inactive angiotensin I to the physiologically active angiotensin II¹⁶. This same enzyme also inactivates bradykinin, a peptide which regulates inflammation, vascular shock and pain perception. Bradykinin has recently been localised to specific neuronal systems in the brain¹⁷. Thus, enkephalinase inhibitors might influence bradykinin and angiotensin as well as

enkephalin.

A role for opioid peptides in mental illness was suggested by preliminary reports of a beneficial effect of the pituitary peptide β -endorphin in schizophrenic and depressed patients¹⁸ and reduced auditory hallucinations in schizophrenics treated with naloxone¹⁹. These findings emerged from uncontrolled studies and await full confirmation in double-blind placebo-controlled experimental designs. Support for effects of opioid peptides in schizophrenia emerges from the positive influence of FK-33824 on hallucinations and overall mental state in a study of eight patients in Denmark²⁰ and in six schizophrenics in Munich (N. Nedopil & E. Ruther, personal communication). In the Danish study patients received intramuscular injection of 1, 2 and 3 mg on three successive days, while in the German study 0.5 mg and 1.0 mg were given in intravenous infusions on two successive days. Unfortunately, neither of these studies was conducted in a double-blind fashion.

One controlled study suggests beneficial effects in schizophrenia of an opioid-related peptide which has no opiate activity. [Des-Tyr¹]- γ -endorphin comprises amino acids 62–77 of the pituitary peptide, β -lipotropin. The absence of an N-terminal tyrosine abolishes affinity for opiate receptors. On the basis of its behavioural effects in rats²¹ [des-Tyr¹]- γ -endorphin was administered to chronic schizophrenics²². In a double-blind placebo-controlled crossover design the peptide in daily doses of only 1 mg dramatically alleviated schizophrenic symptoms. □

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Prostacyclin in blood vessel-platelet interactions: perspectives and questions

from Philip Needleman

BLOOD vessels possess the capacity for the intrinsic synthesis of vasodilator prostaglandins, thereby providing considerable potential for the local regulation of vascular tone¹. Prostacyclin^{2,3} or its stable metabolite 6-keto-PGF_{1 α} ^{4,5} is the primary vascular prostaglandin produced. As prostacyclin (PGI₂) is a potent inhibitor of platelet aggregation *in vitro*⁶ it thereby became a prime candidate for the local vascular regulation of thrombotic events.

Isolated blood vessel preparations rather inefficiently (<5%) convert exogenous arachidonate (by way of the enzyme cyclooxygenase) into prosta-

glandin endoperoxides. However, the prostacyclin synthetase in vascular tissue quantitatively (>80%) metabolises exogenous or endogenous PG endoperoxides into PGI₂. On the other hand, platelets possess a very active cyclooxygenase which readily cyclises arachidonate into endoperoxide which is then enzymatically converted into the potent aggregator and blood vessel constrictor, thromboxane A₂. Bunting *et al.*⁶ and Moncada *et al.*⁷ found that blood vessel segments treated with indomethacin (a reversible cyclooxygenase inhibitor) and stirred with platelet-rich plasma, prevented aggregation in mixing experiments. They therefore proposed the hypothesis that endoperoxides are released from platelets during aggregation and are used by

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the blood vessels to synthesise prostacyclin. Such an exchange of products simultaneously bypasses the inefficient vascular cyclooxygenase and deprives the platelets of substrate for thromboxane synthesis. An implication of such an interaction is that thrombosis and vascular tone may be mediated or modulated by the local balance of the ratio of vascular-prostacyclin/platelet-thromboxane.

An understanding of platelet and blood vessel arachidonate metabolism and interactions has therefore become the focus of considerable research. In this issue of *Nature* (page 66), Hornstra, Haddeman and Don report data which demonstrate that unstimulated or collagen-activated platelets do not seem to release endoperoxides which could be used as substrate for PGI₂ synthesis by vascular fragments. They also observe that indomethacin inhibition of the aortic cyclooxygenase was reversible, and that intrinsic vascular PGI₂ was the culprit that inhibited the platelets in mixing experiments. We recently demonstrated⁸ that exogenous [¹⁴C]-arachidonate added to intact human platelets in the presence of blood vessel microsomes (as a source of prostacyclin synthetase), resulted in the production of [¹⁴C]-thromboxane B₂ only. PG endoperoxides were released from the intact platelets only when thromboxane synthesis was inhibited by imidazole (an antagonist of thromboxane synthetase *in vitro*); the blood vessel microsomes converted the released endoperoxide into PGI₂. Furthermore, incubation of unstimulated or thrombin-activated human platelets which contain [¹⁴C]-arachidonate-labelled phospholipids, with aspirin-treated intact aorta, immediately resulted in adhesion. The only cyclised arachidonate product of this platelet-blood vessel adhesion reaction was labelled thromboxane, while no labelled 6-keto-PGF_{1α} was detectable. However, thrombin stimulation of imidazole-inhibited platelets resulted in the release of platelet-derived labelled PG endoperoxides which were converted to labelled PGI₂ by the vascular prostacyclin synthetase. Comparable results have been obtained by Baenziger *et al.*⁹ (*Cell* in the press) who incubated cultured human arterial smooth muscle

and venous endothelial cells with human platelets in the presence of arachidonate and measured PGI₂ production by bioassay (that is, inhibition of [¹⁴C]-serotonin release from platelets). Significant prostacyclin synthesis from platelet-derived PG endoperoxides by cultured cells pretreated with aspirin was observed only when platelet thromboxane synthesis was inhibited. All these results mitigate against the attractive notion that blood vessels derive endoperoxide from platelets for subsequent conversion to PGI₂ and are thereby protected from deposition of platelets on vessel walls. Fortunately, however, these experiments have unmasked a pharmacological strategy which may enable one to manipulate the platelet-blood vessel interaction to improve the local prostacyclin/thromboxane ratio. This might be useful therapeutically in thrombosis, coronary and cerebral vasospasm and perhaps atherosclerosis. Development of a thromboxane synthetase inhibitor effective *in vivo* could facilitate the release of platelet endoperoxide on which vascular prostacyclin synthetase could then act. As vascular injury initiates platelet adhesion and aggregation, a thromboxane synthetase inhibitor has the potential selectively to generate prostacyclin right at the critical site.

Despite the demonstration of a fascinating range of pharmacological effects, the physiological and pathological role of vascular prostacyclin production and its effect on platelet function still needs considerable clarification. For example, it was initially suggested that vascular endothelial cells were the primary vascular source of the anti-thrombotic PGI₂, and loss of endothelium during injury, therefore, favoured local thrombosis¹⁰. However, subendothelial vascular smooth muscle readily produces prostacyclin under conditions which favour thrombosis¹¹. It is difficult to reconcile the synthesis of PGI₂ by blood vessels with damaged endothelium with the idea that PGI₂ is one of the chief deterrents of thrombosis.

Another thought-provoking platelet-blood vessel interaction was recently reported in *Nature* by Gryglewski *et al.*¹² and Moncada *et al.*¹³ Their discovery suggests that prostacyclin might be continuously synthesised by the lungs and released as a circulating hormone into the arterial blood. However, the actual PGI₂ levels present in the arterial blood must be ascertained in more physiological circumstances. In the above studies the demonstration of arterial PGI₂ levels was in anaesthetised heparinised animals in which the blood was recirculated through a peristaltic pump. By analogy, previous experience with intact animals has demonstrated that high levels of renal pro-

staglandins are produced in anaesthetised, surgically prepared dogs, whereas little if any renal PG production occurs in unanaesthetised trained animals. Indeed, if PGI₂ is circulating in high enough concentrations to interfere with platelet aggregation, then platelet cyclic nucleotide concentrations should be raised in freshly prepared platelet-rich plasma, which is not the case. Furthermore, it should be possible to demonstrate circulating levels of 6-keto-PGF_{1α} (by immunoassay and mass spectrometry for example) in blood drawn from anaesthetised experimental animals or man. The resolution of some of these intriguing questions may help to clarify the physiological and pathological role of intrinsic prostacyclin synthesis in regulating platelet function. □

Iron storage in bacteria

from Pauline M. Harrison

ENTERIC and other bacteria have been known for some time to synthesise low molecular weight siderophore molecules of high iron affinity, which enable them to scavenge the iron they require from the medium, even when it is present in low concentration. The bacteriostatic effects of the iron-carrier in serum, transferrin, and of similar molecules in milk and in egg-white may be due to competition by these molecules with the siderophores for iron. Synthesis of siderophores is regulated by negative feedback mechanisms, which shut off their synthesis when iron is abundant. The need for a means of storing iron in bacteria has not been apparent and until recently no counterpart has been found of the iron-storage molecule, ferritin, which occurs in a variety of eukaryotic species from fungi to man. Now ferritin, or a ferritin-like molecule, has been found in several bacterial species. The molecule characterised by Stiefel and Watt (this issue of *Nature* page 81) as a bacterioferritin was first isolated from the nitrogen-fixing bacterium *Azotobacter vinelandii* by Bulen and coworkers (*Biochem. biophys. Res. Commun.* **54**, 1274; 1973) who described the molecule as a *b*-type cytochrome containing large amounts of non-haem iron and no labile sulphide. Stiefel and Watt now show that this molecule is not only a haem protein, but closely resembles mammalian ferritin in several respects. The combination of both types of iron within a single molecule is most unusual and is not known in ferritins

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of higher organisms. To what extent then are Stiefel and Watt justified in designating their molecule a bacterioferritin?

Ferritin from animal or plant sources is a molecule of potentially high but variable iron content (up to at least 30% by weight or over 4,000 Fe atoms per molecule) held within a protein shell. Its iron is in the form of 'micelles' or microcrystals of hydrous-ferric-oxide-phosphate, which can be seen in the electron microscope without staining or shadowing as particles of about 60 Å diameter. This property of ferritin enables it to be recognised within cells and it has been used as a marker for other molecules tagged to it. It has been shown in several species that iron stimulates the biosynthesis of ferritin: protein subunits assemble to form shells, which accumulate iron. A recent high resolution electron density map of horse spleen apoferritin (Banyard, Stammers & Harrison *Nature* **271**, 282; 1978) shows the molecule as a symmetrical assemblage of 24 subunits each of 18,500 molecular weight with high helix content giving an outer diameter of 130 Å. The structures of several other crystalline ferritins are very similar, but some animal and plant ferritins may be larger. Mössbauer spectroscopy of horse spleen ferritin provides evidence of anti-ferromagnetic ordering within the iron cores at low temperatures, the ordering temperature depending on iron content and particle size.

The 'bacterioferritin-cytochrome' of Stiefel and Watt has an iron content of 13–20% by weight, a subunit weight of 17,000, a molecular diameter comparable to that of animal ferritin and an electron-dense core of 55 Å. Its magnetic susceptibility and temperature-dependent Mössbauer spectra establish it as containing weakly-coupled high-spin Fe^{III} reminiscent of ferritin iron. However, the presence of protoporphyrin IX (one per two protein subunits) is distinctive and the potential required to reduce both forms of iron is more negative than that for horse spleen ferritin. Moreover, the bulk of the Fe^{II} is retained inside the molecule on gel filtration, whereas it is more readily lost from the horse spleen protein, presumably diffusing out of the molecule through channels in the protein shell seen in the electron density maps. Stiefel and Watt have considered the possibility that their 'bacterioferritin' may act as an electron store or as a specific iron-storage depot for the iron-containing

protein nitrogenase.

At a recent meeting on Proteins of Iron Metabolism* Bauminger, Cohen, Levy, Ofer and Yariv of the Hebrew University, Jerusalem, described evidence for the presence of a new type of iron-storage compound in *E. coli* grown on ^{57}Fe -enriched media. Mössbauer spectra of packed cells showed the presence of high spin Fe^{III} with evidence for magnetic ordering below 2.6 K. An iron-rich protein, of approximately 300,000 molecular weight containing electron-dense particles was isolated and gave Mössbauer spectra similar to those of the whole cells, but this soluble protein accounted for only about 1% of the iron content of the whole cells grown on the most iron. These workers consider the pure protein from *E. coli* to be quite distinct from ferritin. They report similar ^{57}Fe spectra from *Proteus mirabilis* and *Mycoplasma capricolum*. This suggests that the requirement for an iron-storage molecule may be universal.

A similar molecule may also be present as a minor component of a freshwater magnetotactic spirillum in which most of the iron (1.5% of the cell dry weight) is present in chains of magnetite crystals (each about 100 nm across), which account for the alignment of these organisms in the geomagnetic field (Frankel, Blakemore & Wolfe *Science* **203**, 1355; 1979).

It is not yet clear how similar the *E. coli* protein is to that from *Azotobacter vinelandii*, although the chromophore in addition to the non-haem iron also seems to be present in the *E. coli* protein. Fortunately both proteins have been crystallised and the results of further structural studies are eagerly awaited as well as those of biochemical studies which may more clearly establish their biological roles and their resemblance to and differences from ferritin. □

*Held at Davos, Switzerland on 17–21 April, 1979.

Errata

In the article 'Acetylcholine receptor clusters' (*News & Views*, **278**, 599; 1979) a line was omitted from the second paragraph, third column, page 599. The sentence beginning on line 12 of that paragraph should read "After the initial stage of cluster formation, the appearance of new hot spots in the absence of a nerve fibre has not been observed on morphologically stable muscle cells."

In the footnote to the article 'Membranes and Parasites' (*News & Views* **277**, 12; 1979) it should be noted that the Special Programme for Research and Training in Tropical Diseases is sponsored by The World Bank, The United Nations Development Programme and the WHO.

Actinide magnetism: an extraordinary tale

from Gillian Gehring

SOME intriguing experiments on the magnetic properties of actinide inter-metallics containing uranium have produced results which cannot be explained by any of the standard theories of magnetism. The most anomalous material is USb (uranium antimonide) which has been extensively studied by Lander *et al.* (Lander *et al. Phys. Rev. B* **14**, 5035; 1976; Lander *et al. Phys. Rev. Lett.* **40**, 523; 1978; Lander *et al. Phys. Rev. Lett.* **42**, 260; 1979) but the behaviour of UN (uranium nitride) which has been studied by Buyers *et al.* (*Proc. Int. Symp. on Neutron Inelastic Scattering*, Vienna, 1978) is similar. However, all the materials AnX where An is uranium or neptunium and X is nitrogen, phosphorus, arsenic, antimony, sulphur, selenium or tellurium have the same or similar magnetic phases (Aldred & Lam, *The Actinides: Electronic Structure and Related Properties I* (eds Freeman & Darby) Academic Press, New York, 1974) so that it is possible that what has been seen in USb and UN is actually characteristic of a whole class of compounds and not just due to some freakish property. The properties of a magnetic material depend on a delicate balance between various interactions and it may be that the particular combination characteristic of the actinides; large spin orbit interaction, variable valance and an apparent tendency for metallic f electrons will call for a totally new theory.

Studies of the magnetic properties of materials have been of great importance in helping our detailed understanding of solid state phenomena. This has been, I think, for two main reasons. The magnetic interactions which are odd under time reversal, split electron degeneracies which cannot be removed by any other means so their explanation is a very critical test of our understanding of a material. Also a very wide variety of experiments may be done. Above the phase transition one can measure the susceptibilities of the system to uniform or varying magnetic fields; close to the transition one sees very pronounced fluctuations in the magnetisation which may exist over large regions of space (that is, involving many crystal sites) and decay only slowly with time; at low temperatures one may study the ordered state and measure the magnetic moment per site as a function of

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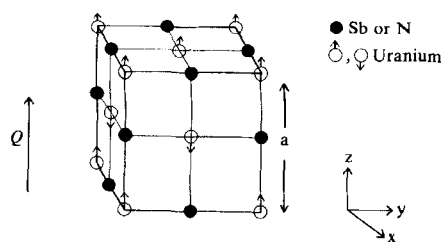


Fig. 1 The magnetic structure of USb and UN for a domain with $Q = \frac{2\pi}{a}[001]$

temperature and also measure the magnetic excitation energies.

The AnX crystals have cubic, NaCl structure; in Fig. 1 the magnetic structure for USb and UN is shown. The spins are arranged in sheets perpendicular to one of the crystal axes (z in Fig. 1) so that all the spins in a given sheet are parallel and adjacent sheets are antiparallel; the magnetic moments are perpendicular to these planes. The moment configuration may be described by

$$m_n = zme^{iQ \cdot R_n} \quad Q = \frac{2\pi}{a}[001]$$

where R_n is the position vector of the uranium site. In an antiferromagnetic crystal it is very useful to specify the Q vector which characterises the order. Above the phase transition the x , y and z directions are all equivalent so there are three possible Q vectors corresponding to ferromagnetic planes perpendicular to the x , y or z direction. However, once the planes have been chosen the spins are constrained to lie parallel to Q . The magnetic moment on the uranium site is $2.82 \mu_B$ for USb; this is comparable with that of the free ion U^{3+} ($J = 9/2$) $3.42 \mu_B$ or U^{4+} ($J = 4$) $3.34 \mu_B$ (Lander *et al. op. cit.* 1976). The magnetic moment distribution on the U sites has been shown to be an oblate spheroid: that is, flattened in the z direction. The transition temperature of USb is 241.2 K which is considerably higher than that of the equivalent lanthanide compound NdSb for which $T_N = 13.6$ K.

The high temperature and critical behaviour of USb and UN have been studied by neutron scattering by Lander *et al.* and Buyers *et al.* Since the ordering which is going to occur at low temperatures is characterised by Q then the system will be expected to show a diverging susceptibility to a magnetic field which has the same alternating spatial dependence, that is

$$h_n = z h_0 e^{iQ \cdot R_n}$$

Such a spatially varying field may be provided by scattering a neutron through wave vector Q . In such an experiment one looks only at one domain because the scattering done at

or near $Q = \frac{2\pi}{a}[001]$ for example, will

arise only from those parts of the crystal which are ordering with ferromagnetic planes perpendicular to z . Above the phase transition the clusters of spins which are ordered are small and they grow as the transition is approached. In both USb and UN the aligned clusters are predominantly in the planes; the correlation length is five times larger in the plane as out of it for USb, for UN it is a factor of three. This is not unusual in layer compounds but USb is cubic so that the neighbouring uranium sites out of the plane are at the same distance as those in the plane. (A very small distortion of less than 0.1% does actually occur at low temperatures). In addition the direction of the spins is also rigidly fixed perpendicular to the planes so that the system has a negligibly small response to a magnetic field which is perpendicular to Q . Systems in which the moment may only point up or down are known as Ising systems; they are characterised by a slow relaxation time above T_N , as is found in USb and UN, and their magnetisation tends to zero at T_N like $(T_N - T)^{0.3125}$ (in USb the exponent was found to be 0.32 ± 0.02). Again this highly anisotropic behaviour has not been observed before in a cubic compound.

The excitation spectrum at low temperatures is even more anomalous. In ordered magnets the elementary excitations are spin waves. These have been seen in systems, such as MnF_2 or $NdSb$, in which the electrons which are responsible for the moment are localised on the magnetic site, and in itinerant ferromagnets such as metallic nickel. The form of the excitation and the way in which its energy depends on wavelength are sketched in Fig. 2 for two extreme models. In the Ising model the angular momentum component in the z direction on one site has been reduced by one unit. Although this is a localised excitation we may consider a linear combination of these states with wave vector k but the energy will be independent of k . This situation arises if the angular momenta are constrained so that only the z component may be non-zero. The opposite extreme is the Heisenberg model. In

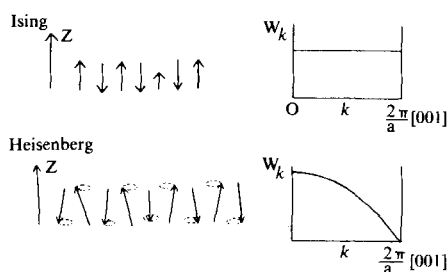


Fig. 2 Sketch of the form of the excitations in Ising and Heisenberg systems with the type of dispersion relation— w_k versus k plot.

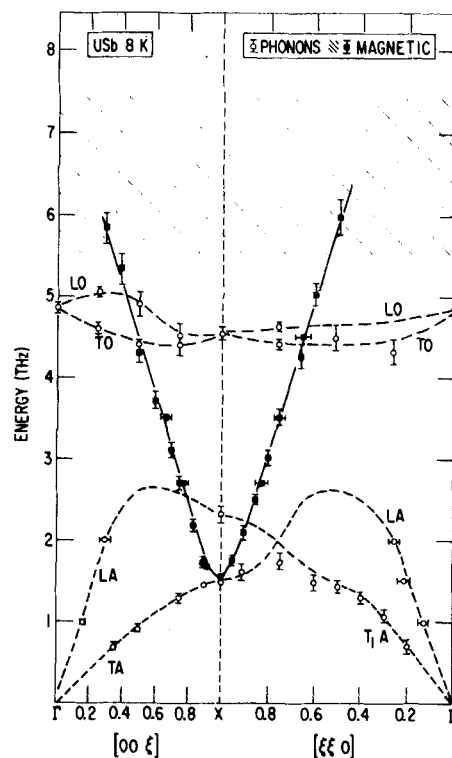


Fig. 3 The dispersion curves for USb; energy plotted against wave-vector transfer Q (in units of $2\pi/a$). The dashed lines represent the phonon dispersion and are based on the measured open points as well as on our knowledge of phonons in NaCl structures. The magnetic modes are represented by solid squares (the collective excitation) and the hatched area (excitonic level). (From Lander *et al. Phys. Rev. Lett. op. cit.* 1979).

this case all the components of angular momenta may be allowed to be non-zero and an exchange coupling of the form $\mathcal{J}(nm)(J_n^x J_m^x + J_n^y J_m^y)$ can transfer the excitation from site n to m . The energies of the excitations vary strongly with k . An intermediate case will arise if there is some anisotropy energy favouring the z direction and the excitation spectrum will show some dispersion. As the temperature is raised many moment deviations occur; the effect of this is that the energy of the excitations fall (approximately with the magnetisation) and they become less well defined as they scatter off each other. These excitations may be created by a transverse time-dependent magnetic field: this is because the operators which take state $|J, M_J\rangle$ into $|J, M_J \pm 1\rangle$ are $J^{\pm} = J^x \pm iJ^y$. At low temperatures a time-dependent magnetic field in the z direction has very little effect because it cannot create these spin waves. Near to T_N there is a diffusive response to a time-dependent field h_z . This behaviour of the transverse and longitudinal excitations has been observed in essentially all ionic and metallic magnets.

The behaviour in USb and UN is quite different. Neither crystal shows

any response to a transverse magnetic field. In USB there is a well-defined excitation for $T < T_{N/2}$ which has a lot of dispersion as shown in Fig. 3. Its energy increases slightly as the temperature is raised, it is characteristic of a system with equal strengths of interactions between sites in and perpendicular to the planes, and it is excited only by a field in the z direction. In UN there is some evidence of a similar excitation but it becomes very strongly damped away from the point at which it has its minimum energy. Incidentally the minimum energy of this

excitation appears to coincide exactly with the zone boundary energy of the transverse acoustic phonons in both compounds.

There is no way in which the well known theories of localised magnetism nor the theories of itinerant magnetism which work for the transition metal elements and alloys can account for these features. Something really new will be needed in which the interesting but difficult question of localised *versus* itinerant description of electrons will be raised again but this time including spin orbit coupling as a large effect. $\square$

Bacterial chemotaxis and protein carboxymethylation

from Gerald L. Hazelbauer

MOTILE bacteria migrate along chemical gradients toward higher concentrations of some compounds (attractants) and away from higher concentrations of others (repellents). A number of recent studies of the biochemistry of chemotactic behaviour in *Escherichia coli* and *Salmonella typhimurium* document the central importance of carboxymethylation of a group of membrane proteins. This constitutes significant progress in the understanding of the bacterial sensory response system and is particularly tantalising since protein carboxymethylation has been detected in a large number of mammalian tissues (Kim *et al.* *J. Neurochem.* **24**, 625; 1975; Diliberto & Axelrod *J. Neurochem.* **26**, 1159; 1976) and at least in some cases the reaction appears to be correlated with cellular response to chemical stimuli (Diliberto *et al.* *Proc. natn. Acad. Sci. U.S.A.* **73**, 4050; 1976; O'Dea *et al.* *Nature* **272**, 462; 1978).

An *E. coli* cell swims in straight lines punctuated every few seconds by episodes of uncoordination, called tumbles, that result in new, randomly-chosen directions of swimming. A change in concentration of an active compound over either distance (spatial gradient) or time (temporal gradient) results in an alteration in tumble frequency. Favourable changes suppress tumbles; unfavourable ones induce them. Thus a cell makes net progress along a spatial gradient by longer path lengths in favourable directions. Coordinated swimming occurs when the left-handed helices of bacterial flagella rotate counterclockwise and tumbling occurs when they rotate clockwise. The sensory system in-

fluences migration by controlling the direction of flagellar rotation and the probability of reversals. Sensitivity to active compounds is mediated by specific receptors, some of which have been identified as particular cell surface proteins.

The bacterial response to a gradient is transient. Addition of an attractant to a cell suspension results in an immediate suppression of all tumbles and addition of repellent induces continual tumbling. However after a time ranging from seconds to several minutes, depending on the compound and the magnitude of the gradient, the cells resume their initial behavioural pattern of swimming and tumbling even though the attractant or repellent is still present. Thus bacteria, like many sensory cells in higher organisms, adapt. An important body of work primarily by Goy, Springer and Adler establishes a convincing correlation between adaptation and methylation or demethylation of the methylaccepting chemotaxis proteins (MCPs), polypeptides of approximately 60,000 molecular weight that reside in the bacterial cytoplasmic membrane (see Goy & Springer in *Taxis and Behavior* (Ed. Hazelbauer) Chapman and Hall, 1978 for a summary of that work).

MCPs are methylated by donation of methyl groups from S-adenosylmethionine to form glutamyl 5-methylesters (Kleene *et al.* *J. biol. Chem.* **252**, 3214; 1977; Van Der Werf & Koshland *J. biol. Chem.* **252**, 2793; 1977). The reaction is catalysed by a soluble carboxymethyltransferase apparently specific for MCPs (Springer & Koshland *Proc. natn. Acad. Sci. U.S.A.* **74**, 533; 1977). Mutations in *che* genes generally perturb tactic behaviour. In both *E. coli* and *S. typhimurium* one *che* gene appears to produce the methyl transferase and another a demethylase

(Stock & Koshland *Proc. natn. Acad. Sci. U.S.A.* **75**, 3659; 1978).

Goy *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 4964; 1977) measured the levels of methylation of MCPs during tactic behaviour by *E. coli*. Unstimulated cells exhibit a basal level of methylation. On stimulation the extent of methylation increases (for favourable changes) or decreases (for unfavourable changes), over a period that corresponds to the adaptation time, to a new level which is maintained as long as the chemical environment remains unchanged. The new level of methylation is a function of the magnitude of the stimulus. The straightforward interpretation of these observations is that adaptation is the result of methylation. That predicts that cells unable to methylate MCPs should be unable to adapt. Springer *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **74**, 183; 1977) had previously shown that methionine-starved cells, which should lack the S-adenosylmethionine necessary for methylation, do not adapt but that addition of exogenous methionine subsequent to tactic stimulation allows a delayed adaptation to proceed. Recent studies of *cheX* mutants of *E. coli* by Parkinson and Revello (*Cell* **15**, 1221; 1978) and Goy *et al.* (*Cell* **15**, 1231; 1978) demonstrate that cells in which methylation of MCPs is defective as the result of a mutational block are also defective in adaptation. *CheX* mutants exhibit low levels and aberrant patterns of MCP methylation in both unstimulated and stimulated cells, a phenotype consistent with the *cheX* product being the methyltransferase (Stock & Koshland *op. cit.*). In a constant environment the mutants almost never tumble but temporal gradients of repellents induce tumbling. Using cells induced to tumble, both laboratories showed that temporal gradients of attractants suppress tumbles. The striking defect in the mutants is that they exhibit greatly extended adaptation times. In fact *cheX* cells subjected to relatively strong stimuli maintain the stimulus-induced behaviour of continual tumbling or exclusively smooth swimming for as long as the investigator cares to wait (36 h in some cases). It appears that *cheX* mutations do not significantly affect the ability of cells to be excited by tactic stimuli but only the ability to adapt to them. As the authors suggest it seems likely that the smooth-swimming phenotype of *cheX* strains reflects an inability to adapt to previously encountered stimuli rather than a defect in the tumbling mechanism itself. In any case the observations that two independent blocks on methylation of MCPs result in the same adaptation-defective phenotype provides strong evidence that the reaction is central to

adaptation.

In addition it is clear that the MCPs are critically important for the transduction of tactic signals from receptors to the flagellar motor. Springer *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **74**, 3312; 1977) and Silverman and Simon (*Proc. natn. Acad. Sci. U.S.A.* **3317**; 1977) both demonstrated that two different genes, *tsr* and *tar*, code for MCPs. Mutations in *tsr* eliminate response to one set of stimuli (serine, some other amino acids and some repellents) and those in *tar* to a complementary set (aspartate, maltose and some repellents). Thus excitation mediated by one group of receptors requires the *tsr* function and by another group requires *tar*. The division extends to the methylation reaction. *tsr*-mediated stimuli result primarily in methylation of MCP I, the product of *tsr*, and *tar*-mediated stimuli cause methylation of MCP II. Both sets of authors suggest that MCPs I and II together accept tactic signals from most if not all receptors and thus represent the final stage of signal transduction.

That suggestion may require some revision since Kondoh *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **76**, 260; 1979) describe an MCP III which is methylated upon stimulation by galactose,

ribose or their analogues and is independent of *tsr* and *tar*. This is particularly interesting since the acceptor of signals from galactose and ribose receptors is thought to be the product of the *trg* gene. Mutants in *trg* do not respond to gradients of ribose or galactose but respond normally to other attractants (Ordal & Adler *J. Bact.* **117**, 517; 1974; Hazelbauer & Harayama, *Cell* **16**, 617; 1979). MCP III is not methylated in *trg* mutants but it is not yet clear if the *trg* product is the MCP III protein or instead is a first-stage transducer that funnels into MCP III. In the latter case one might expect to find analogous first-stage transducers for other receptors. The former possibility suggests that MCP I and II should interact directly with the receptors they serve.

It seems evident that much effort will now go into the purification and characterisation of MCPs. The proteins must interact with receptors or first-stage transducers on one hand and with the enzymes of methylation and demethylation and perhaps additional *che* products on the other. An understanding of the mechanisms of signal transduction and adaptation will require a detailed elucidation of those interactions. □

News from PETRA and DORIS

from David J. Miller

THE new electron-positron storage-ring PETRA has now been working for about 6 months at the DESY laboratory near Hamburg and the first physics results have just been reported. They are interesting but not unexpected. Three detectors were used, each manned by a different international collaboration of universities and national laboratories. All see the same sorts of effects, but with different sensitivities (see for example *DESY preprint 79/11* and papers in recent issues of *Phys. Lett.*). Data have been collected at total energies of 13 GeV and 17 GeV. There is evidence for the increase, as energy is increased, of the tendency for strongly-interacting particles (hadrons) to be produced in a pair of 'jets'. A jet is a cluster of particles all moving in roughly the same direction and all generated, according to present theories such as quantum-chromodynamics (QCD) (see *News & Views* **277**, 349; 1979) by one fundamental parent—a quark or a gluon. In QCD the underlying interaction is supposed to be between quarks and gluons; the quarks behaving like particles when within a femtometre of each other but needing to convert

themselves into pions and other genuinely free hadrons when they try to separate. Fast gluons are expected to form jets in a similar way. As well as their clear two-jet structure, the PETRA data on the interaction rate and on the number of particles produced conform to the pattern which has been observed at lower energies with the SPEAR storage-ring in California and up to 10 GeV with the DORIS rings at DESY.

All hadron production by electron-positron annihilation can still be explained by production of pairs of quarks and antiquarks from the set *u*, *d* (the quarks in normal matter such as protons or neutrons), *s* (the strange quark), *c* (the charmed quark) and *b* (the heavy 'bottom' quark). Since the production process goes by way of a single intermediate photon the contribution due to each type of quark depends on the square of its electromagnetic coupling—that is, the square of its charge. The bottom quark appears to have 1/3 times the electron charge so it is produced at only one quarter of the rate at which the 2/3-charge *c* quark is produced. The threshold energy for *c* anti-*c* production is about 3.8 GeV. Below that the narrow *psi-J* and *psi'* 'charmonium'

bound-states are seen. Above 3.8 GeV there is a significant increase in the rate of hadron production. Then, as more and more spare energy becomes available, the two quarks have to get faster and jets are formed. Just below 10 GeV the pattern seems to be repeating the narrow *upsilon* and *upsilon'* 'bottomonium' states being found (see *News & Views* **273**, 705; 1978). At the new PETRA energies there is just a suggestion of extra hadron production due to *b* anti-*b* production and a slight check in the rate of increase of two-jet properties, though the underlying trend is dominated by the lighter quarks with 2/3 charge. The real interest over the next few months will come as energies are increased from 17 to around 30 GeV. Many theorists expect a heavy 2/3 charge quark called *t* ('top') which should give another clear step in the rate of hadron production along with a sudden dilution of the two-jet structure. When such changes are seen it will be possible to search back in energy to the *t* anti-*t* threshold and then to look for *psi* or *upsilon*-like 'toponium' states just below it.

One of the collaborations at PETRA, using the PLUTO detector, has reported another important result which especially interested physicists who visited DESY in the first week of April to continue the studies for a very high energy European electron-positron collider (LEP) (see *News & Views* **275**, 482; 1978). The PLUTO group has seen 100 events caused by the collision of pairs of virtual photons, one contributed by the electromagnetic field of an electron in the electron-beam, the other from the field of a positron in the opposed beam. Such events are predicted to be much more frequent at higher beam energies and they may form an unpleasant background to the important single photon annihilation processes. But they also open a new window onto processes which have not yet been studied so their observation at PETRA will encourage those who have been planning special experiments to look at these reactions.

The most important recent result comes, however, not from PETRA but from DORIS. After last year's announcement of their observation of the *upsilon*, the PLUTO group moved the detector to PETRA but continued to analyse data from DORIS. These show that hadron production by way of the *upsilon* seems to be dominated by three jets rather than two. This has been demonstrated by statistical analyses of particle distribution (see *DESY preprint 78/71*) and they have now reported an 'energy-flow' diagram which shows clear signs of three-jet structure which they claim cannot be

reproduced by any other simple mechanism. Some theorists are hailing this result as the proof that gluons exist, though many people are not fully convinced.

If the upson is produced by way of a single photon then it must have negative charge-conjugation parity (C-parity). Since the upson is too light to decay to the b -anti- b continuum it can only decay to quarks if the weak interaction turns a b into a lighter c quark, but that is a very slow process. In the meantime there is predicted to be a high probability that it can decay into gluons. But gluons are like photons in a number of ways. In particular, they also should have negative C-parity, so the upson can only conserve C-parity if it decays to an odd number of gluons. Energy-momentum conservation forbids decay to one gluon and the phase-space probability is small for five gluons or more. Sceptics claim, correctly, that other three-jet models can be constructed, but QCD does predict that three gluon jets should be seen and the PLUTO results must therefore be taken seriously as at least circumstantial evidence for the existence of gluons inside hadrons. $\square$

Ten years after . . . a decade of lunar science

from C. T. Pillinger

THE 10th anniversary meeting of the Lunar and Planetary Science Conference* met under the shadow of recent budgetary constraints imposed by the US Congress. The growing 'planetary' nature of the conference was marked; indeed discussion on new data from lunar soils has diminished substantially from its originally dominant position. Data from new planetary missions, meteorites and studies of fundamental processes on the Moon attract more interest from today's planetologist.

A highlight of the conference was the presentation of new results from the Viking, Voyager and Pioneer missions. A short 3-D film of Mars, created from elegant computer techniques by E. Levinthal (Stanford University), preceded spectacular close-up pictures of Jupiter and the Gallilean satellites, providing exciting new venues for geological speculation. The volcanic activity on Io, and apparent tectonic effects on Ganymede, will no doubt provide the basis for much discussion at future meetings. The composition of the atmosphere of Venus, with its apparently 'solar' rare gas

abundance (see *News & Views* 278, 777; 1979), leads one to speculate as to how radically our current ideas of planetary formation may have to be revised over the next 10 years.

As pointed out by S. R. Taylor (Australian National University, Canberra), 10 years study of the Apollo samples has failed to achieve consensus on the origin of the Moon, although considerable constraints can be inferred. Existing disagreements continued over the criteria for estimating siderophile element abundances indigenous to the highland crust and the whole Moon. Delano and Ringwood (Canberra) disputed the elemental correlations calculated by the group of E. Anders (University of Chicago). The importance of the argument is the validity of the assertion by Ringwood that the Moon was derived from the Earth's mantle after formation of the Earth's core, as opposed to condensation as a discrete body. A third position was adopted by Wanke *et al.* (University of Mainz) who, on the evidence of Co/(Fe + Mg) ratios, favoured a genetic relationship between the Earth and Moon.

Continued studies of lunar drill core samples have clarified ideas concerning reworking and deposition processes in the lunar surface layer, known in lunar parlance as 'the regolith'. Soil exposure indices have been based on a number of irradiation effects and petrographic particle size data. However, the relative importance of erosion compared with deposition processes (for example sputtering, impact volatilisation and deposition, and micrometeorite erosion) continues to be the subject of lively debate.

Wieler *et al.* (University of Zurich) showed that 0.5 billion year old soils had similar solar rare gas ratios to the present; no information on the solar wind flux in the past was forthcoming, although various workers have postulated an increased flux of varying isotopic composition in earlier irradiation. The magnitudes of the postulated changes seem to be at variance with existing models of solar processes.

Meteoritic studies have benefited from techniques developed for the lunar programme, and the growth of such investigations was evident. The Allende meteorite in particular has been subjected to intensive study. The Caltech group presented a detailed study of a new hibonite-rich inclusion (HAL); hibonite is considered to be one of the highest temperature major element condensates from the solar nebula. This inclusion consisted of an unusually pure hibonite core and several rims of lower-temperature

phases. Comparison with other Allende Ca, Al-rich inclusions implied a large ^{26}Mg anomaly; however, this was not found, suggesting a low ^{26}Al content for HAL. Ca isotope shifts in all phases were found to be large but uniform, and small nonlinear effects attributed to a nuclear origin. HAL thus qualifies as a new Allende isotopically-anomalous inclusion. Jessberger *et al.* (University of Heidelberg) reported a very high $^{39}\text{Ar}/^{40}\text{Ar}$ apparent age of over 5 billion years, which can either be interpreted as a very old component, or a possible ^{40}K isotopic enhancement. The latter possibility should be resolved by work now under way in Mainz. The existence of very old inclusions (> 5 billion years) would imply that either these inclusions originated from outside the condensing solar nebula, or that the currently accepted age of the Solar System of 4.5 billion years needs to be re-assessed.

Strongly nonlinear kinetic isotope fractionations of the three stable isotopes of oxygen have been produced in the laboratory. G. Arrhenius (La Jolla) suggested this effect as another source of the nonlinear isotopic behaviour, to complement the nucleosynthetic processes. The latter were proposed to explain the original discovery of such anomalies in oxygen by R. N. Clayton in 1973.

Recent and previous discoveries of isotopic anomalies place constraints on possible models of the origin of the Solar System. However, considerable latitude is still available, as a number of apparently contradictory models were discussed at length mainly by D. D. Clayton (Rice University) and A. Cameron (Harvard University).

An interesting new sample collection method, which may be important to future research, is the 'cosmic muckrake' of Brownlee and others. Using a magnetic rake dragged across the ocean floor, large quantities of extraterrestrial material can be recovered. In a typical day's operation, the device processed $\sim 10^7$ kg of sediment, and separated about half a million particles, many of which were identified as extraterrestrial. Debris from nearly every type of object which has collided with Earth over the past 10^8 years might be collected.

The few contributions mentioned outline some promising research emerging in planetary science. The emphasis on the Moon a decade ago has given new impetus to the older science of meteoritics; additionally, new information from hitherto inaccessible objects is now becoming available. $\square$

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*Held at the Johnson Space Center, Houston on 19-23 March.

review article

Games parasites play: how parasites evade immune surveillance

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Parasites have evolved an extraordinary variety of mechanisms for surviving in the face of the natural and acquired immune responses of their hosts. A selection of the mechanisms serves to illustrate the challenge involved in developing strategies for immunising against parasites.

THE games parasites play are, in fact, a serious matter (see refs 1–11 for reviews). It is estimated that between 500 million and 1,000 million people, primarily in the developing countries, suffer from tropical diseases and parasitic infections. Table 1 indicates current estimates on the prevalence of some parasitic diseases. In addition to the diseases of man, millions of cattle die of parasitic diseases each year (Table 2) and at least 700 million are estimated to be at risk. In Africa alone, 7 million square kilometres of grazable land capable of supporting 120 million head of cattle remain largely unproductive chiefly because of two parasitic diseases—trypanosomiasis and East Coast fever.

There is currently a resurgence of interest in tropical diseases, and in the possibility of providing immunological protection against many of them. The challenge to the immunologist, in trying to engender resistance or immunity to these parasites, is to improve the natural capability of the host's immune system, given that the survival of parasites and the extent of their ability to live in the host species reflect the fact that they have evolved mechanisms for surviving in the face of the best the body has to offer in the way of natural and acquired immunity.

In the simplest of terms, the general ground rules of the game may be sketched as follows: If parasites totally eluded the immune response and were sufficiently virulent, they would kill their hosts on whom their survival depends and preclude their own survival. Conversely, if they were too easily destroyed by the immune response, their survival would be similarly jeopardised. Consequently, immunity and escape from surveillance are relative phenomena which exist in balance and tension.

My intention here is to outline some of the mechanisms currently put forward to explain escape from surveillance. From this rapidly accumulating knowledge there should arise some new approaches to the prevention of parasitic diseases. It is to be expected that as these approaches are applied, they will be recognised by the parasites as selective pressures to be overcome, and they will devise even more sophisticated mechanisms for eluding the host's immune response. But one may hope that the time frame of evolution will be a great deal longer than the time frame required to bring most infectious diseases under some control.

Old-time genetic engineering

In West Africa, there is considerable variability in susceptibility among various breeds of cattle to infections produced by *Trypanosoma congolense*. Cattle introduced to Africa from Europe during the time of the Roman Empire as well as native zebu cattle are highly susceptible to this parasite. Indigenous West

African short-horned cattle such as the n'dama and muturu seem to be naturally much more resistant¹². This is an example of what is generally termed 'natural immunity'. The spectrum of natural immunity is illustrated by the fact that there can be some parasites such as *Trypanosoma b. brucei* and *Trypanosoma cruzi* within a single classification which have enormous host ranges and are capable of infecting and growing in virtually all domestic mammals; others, such as *T. simiae*, can produce fulminating infection in only a few, apparently unrelated, species such as monkeys and pigs; and there may be others which produce infections with more limited host range, such as the exclusive infection of cattle by *Theileria parva* or certain species of monkeys by the monkey malaria agent, *Plasmodium knowlesi*. The mechanisms underlying these host restrictions remain largely unknown; yet were such mechanisms understood, it might be possible to learn much about the parameters which govern host-parasite interactions.

In spite of the current enthusiasm about immune response (Ir) genes, in a wide variety of experimental bacterial, viral and parasitic infections studies in inbred mouse strains, it has not yet been established that natural host resistance is primarily dependent on genes associated with the major histocompatibility complex. Within a host species, however, there may be striking genetic differences in susceptibility to parasitic infection. This is perhaps best illustrated by studies on two protozoa that cause quite different diseases in man—*Leishmania donovani*, the causative agent of visceral leishmaniasis or kala-azar and *Trypanosoma cruzi*, which produces Chagas' disease in Latin America. When various inbred mouse strains were studied for susceptibility to *L. donovani* by Bradley¹³, C3H/He mice seemed to be most resistant and C57B1/6, most susceptible. By appropriate crosses it was possible to show that there was a single major autosomal dominant gene which controlled resistance, and that this gene was not H-2-linked. Studies in our

Table 1 Some parasitic diseases in man

	Estimated prevalence
Hookworm	700,000,000
Trachoma	400,000,000
Schistosomiasis	200,000,000
Malaria	150,000,000
Filariasis	200,000,000
Chagas' disease	12,000,000
Sleeping sickness	Unknown
Leishmaniasis	Unknown

laboratory on resistance to *T. cruzi* in the same strains of mice, revealed an almost reciprocal pattern of susceptibility¹⁴. Here the C3H is susceptible and the C57B1, relatively resistant. Again we have found resistance to be unlinked to H-2. It is obviously of great interest to pursue the mechanisms by which these major autosomal genes control resistance. In Bradley's studies, there was some evidence that an H-2-linked gene exerted a secondary influence on resistance following infection. In this regard studies of patterns of the major human histocompatibility complex (HLA) antigens in Sardinia by Piazza *et al.*¹⁵ showed significant differences in the HLA-B locus antigens between inhabitants of the highlands, an area in which malaria was essentially non-existent, and of the lowlands, an area in which malaria flourished until recently. This suggests that there may have been a selection for certain HLA-B-linked, possible Ir gene functions by the malarial parasite.

The converse situation, in which the outcome of infection is modified by the genetics of the parasite, is illustrated by three extraordinarily different disease entities of man in three different parts of the world caused by *Leishmania*, a genus of protozoan parasites which grows exclusively in macrophages in man and certain animal reservoir hosts. *Leishmania donovani* causes the systemic, progressive non-healing disease, visceral leishmaniasis, known in South Asia as kala-azar; *Leishmania tropica* causes Oriental sore found mostly in the Middle East and which is a local, cutaneous, self-healing lesion; and *Leishmania brasiliensis*, which causes mucocutaneous leishmaniasis in Central and South America with its most fulminating form known as espundia, a disease in which the parasite destroys the soft tissue and literally makes its victims faceless. Our lack of knowledge about the genetic control of pathogenesis makes it impossible to understand how three such different diseases can be produced by related organisms that grow in the same cell type. Nor why the same organisms inhabiting different host species produce different patterns of disease than in man.

How parasites change their spots: antigenic variation

There is no more striking example of successful adaptation of a parasite to the host's immune response than that exhibited by African trypanosomes, which live entirely extracellularly, particularly in the blood stream and lymph of ungulates or man where they cause sleeping sickness. As long ago as 1907 Massaglia (see ref. 16) observed that following infection through the bite of the tsetse fly, the numbers of parasites in the blood fluctuated periodically. He suggested that this succession of parasitaemia, remission and recrudescence was due to the destruction of trypanosomes by host antibody and the emergence of parasites of different antigenic constitution. The course of these waves of parasitaemia in a single patient described in 1910 is illustrated in Fig. 1. Human sleeping sickness is caused primarily by two subspecies of *Trypanosoma brucei*, namely *gambiense* and *rhodesiense*. Each wave of parasitaemia is followed by the production of antibodies, generally agglutinins or lysins, which are specific for an individual variant, that is the antigen type of the previous wave of parasitaemia. Antigenic variation has also been described in the genus *Plasmodium*, which causes malaria, and in *Babesia*, tick-borne parasites causing a malaria-like disease.

What are the possible genetic and regulatory mechanisms which could explain antigenic variation? The simplest hypothesis would be that most infections contain a mixed population of parasites comprising several strains, and as the predominant

strain grows and is killed, there is simply outgrowth of another variant type present in the original inoculum. This hypothesis was essentially disproved by experiments in which single trypanosomes were cloned and maintained in immunologically compromised animals and antigenic variation was not seen. When transferred to conventional animals, even though the entire population was derived clonally from a single organism, antigenic variation emerged¹⁷.

A second hypothesis invokes mutation, possibly involving expression of small determinants in the molecule which are immunodominant and which could vary randomly to elude the immune response. A mutation frequency of 1.2×10^{-5} would explain the appearance of variants in a clone at 3–4 day intervals. But Gray showed that antigens of a clone tend to appear in an apparently programmed sequence¹⁸. Thus, display of variant antigens A-B-C-D-? appears to be non-random, and passage of variants at any stage back through the tsetse fly results in reversion to the basic antigenic variant types of the strain. It is difficult to explain a programmed sequence on the basis of simple random mutation.

The mechanism believed to be most relevant to African trypanosomes is adaptive phenotypic variation, in which the parasite has a finite number of genes which can code for its surface antigens and which it can switch on and off in regular sequence. As gene activation and translocation is one of the central problems in molecular biology, the African trypanosomes provide an extraordinarily interesting model for study.

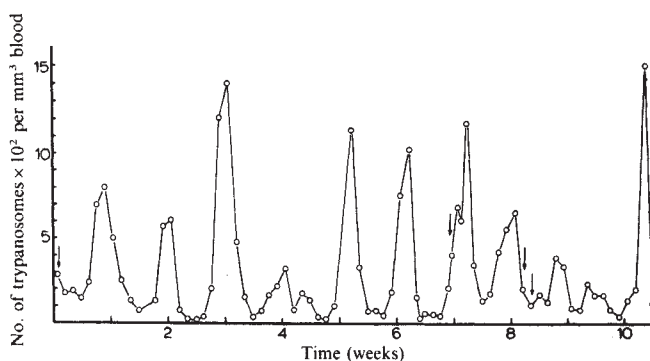


Fig. 1 Parasitaemia occurring in a patient with African trypanosomiasis. From Vickerman¹⁶

What is the nature of the antigens that vary? Cross^{19,20} has demonstrated that the outer surface of *Trypanosoma b. brucei* contains a coat or glycocalyx that is composed principally, if not exclusively of the variant-specific glycoprotein antigen. This antigen has a MW of 67,000, and constitutes 10% of the entire cell protein and 30% of the soluble proteins. The variant glycoprotein molecules contain between 7 and 17% carbohydrate attached to the portion of the molecule associated with the plasma membrane. About 7×10^6 molecules are found per trypanosome, a number probably sufficient to cover the entire surface of the parasite, and it is possible that this coat, which projects 12–15 nm from the plasma membrane, may serve as a spacer or barrier sufficient to prevent antibody to common membrane antigens from ever reaching the plasma membrane and lysing the organism. Amino acid sequencing of the N-terminal ends of four isolated purified variant glycoproteins revealed essentially no structural homologies. Each had a unique sequence, and there was no obvious evidence for deletions or frameshifts, thereby apparently eliminating the simple mutational hypothesis for antigenic variation.

Does antibody select or induce the antigenic variants? As early as 1909 Ehrlich found that trypanosomes incubated with homologous antiserum and then injected into mice produced a parasitaemia of a different variant type. As mentioned before, passage of clones of trypanosomes or variants in immunologic-

Table 2 Some parasitic disease of cattle

	Estimated annual mortality
African trypanosomiasis	3,000,000
Anaplasmosis	1,000,000
East Coast fever	500,000
Babesiosis	250,000

ally incompetent animals results in clonal fidelity. Gray²¹ was able to perturb the programmed antigenic succession in the rabbit by passively protecting animals against four variants of a strain and then challenging with that strain. The rabbits then produced antibody to a fifth variant when challenged with the original strain.

It has become possible for the first time to cultivate the bloodstream form of the African trypanosomes *in vitro*²² using a bovine cell feeder layer, and it is now possible to analyse *in vitro* molecular mechanisms of genetic and regulatory control underlying antigenic variation. Doyle *et al.*²³ followed 19 clones of a variable antigen type of *T. b. brucei in vitro*, and, in the absence of antibody, observed new variants arising spontaneously in nine clones, with a frequency of approximately 10^{-3} – 10^{-4} . In the absence of immunoselection *in vitro*, the ability to detect variant types was related to their ability to outgrow the initial population, suggesting that two mechanisms of selection for variants are likely to be operative *in vivo*, altered growth rates and immunoselection. It remains to be established whether antigenic variants from a clone occur randomly or not, and whether antibodies affect the variation frequency or merely select for spontaneously occurring variants. Nevertheless, the high frequency of spontaneous variants observed in these *in vitro* studies can be considered to support mutational hypotheses for antigenic variation, as the accumulation of large numbers of mutations in the genes coding for surface antigen could lead to production of variants of markedly differing surface antigenicity and amino acid sequences.

As another possible mechanism of antigen modulation it is important to consider the possibility that parasites can 'cap' and shed their surface antigens to elude the immune response. Doyle *et al.*²⁴ have shown that within minutes after exposure of leishmania to fluorescent antibodies at 37 °C, the parasites shed their surface antigens and become refractory to the effects of immune sera and complement. It is not known whether capping occurs *in vivo* and represents a significant mechanism for escape of protozoa from immune attack.

Antigenic mimicry and concomitant immunity

While the term 'concomitant immunity' is most familiar in the context of a tumour-bearing animal in which an autochthonous transplant of the primary tumour is rejected while the primary tumour grows progressively to kill the animal, there is a precisely analogous phenomenon which occurs during infection with a number of parasites. Concomitant immunity²⁵ is a form of acquired immunity in which the established parasitic infection persists long after resistance has developed against a challenge infection. In contrast, 'premunition' is used to describe the situation in which infection is suppressed although not eliminated by the host²⁶.

Concomitant immunity is well documented in schistosomiasis, a chronic disease caused by the trematode genus *Schistosoma*. *S. mansoni* and *S. japonicum* reside in the portal and mesenteric systems and produce a hepatosplenic disease. *S. haematobium* causes a disease of the urinary tract. Adult schistosome worms can reside in the mesenteric venules for many years with little evidence to indicate that they stimulate any immune response at all. However, if adult schistosome worms are transferred to portal veins of monkeys which have never experienced the immature migrating forms, the monkeys become capable of resisting infection when challenged with the immature larval form (cercaria) even though they could not reject the transferred adults²⁷.

How can this type of concomitant immunity be explained? One possibility is that the parasite, by natural selection, has evolved some antigenic determinants similar or identical to those of the host. One difficulty with this hypothesis, at least with respect to schistosomes, is that the organism can live in a variety of mammalian hosts, and it is unlikely that an organism would have evolved major antigens common to all hosts. An alternative hypothesis, originally suggested by Smithers and Terry²⁵ is that schistosomes adapt to the range of hosts in which they live

by appropriating some host molecules onto their surface layer or tegument. This has been amply demonstrated for *S. mansoni*²⁷. Worms grown in both mice and gerbils were transferred either to normal rhesus monkeys or to monkeys immunised against the erythrocytes of mice or gerbils. The adult worms transferred to normal monkeys resumed their egg laying and were viable after about 5–6 weeks. Adult worms transferred from mice to monkeys immunised against mouse erythrocytes failed to establish themselves and were killed. But monkeys immunised against mouse erythrocytes failed to inhibit the growth of adult worms grown in gerbils. These experiments suggested that the worms grown in mice had acquired mouse antigens on their surface.

In order to explain concomitant immunity, it is necessary to argue that the host antigens are acquired by the adult worms but are not found on the immature forms, as the immune response of the chronically infected host can kill the immature forms. Clegg *et al.*²⁸ demonstrated that the most immature form, the cercaria, is not killed by the monkeys immunised against mouse erythrocytes, and hence does not appear to have host antigens, but that by 7 d the intermediate stage of the parasite, the schistosomulum, has already acquired host antigens.

What are the host antigens acquired by these organisms? Schistosomula cultured with human type A, type B or type O erythrocytes have been shown to acquire A, B and H blood group antigens respectively on their outer layer²⁹. Blood group Rh or MN determinants are not found on the schistosomula but several other host serum proteins have been found. Most exciting is the recent evidence of Sher *et al.*³⁰ that schistosomula can acquire mouse histocompatibility antigens, the major determinants of immunological recognition of 'self', and this offers an exquisite mechanism for mimicry of 'self'. Intriguing as these data are, there is as yet no conclusive evidence establishing that 'mimicry' is the mechanism primarily responsible for the failure of adult worms to be rejected by the host.

Learning to live in macrophages

From the time of Metchnikoff, macrophages have been described as highly phagocytic scavenger cells capable of killing and degrading a wide variety of microbes. Nevertheless a number of microorganisms including *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and protozoa of such genera as *Toxoplasma*, *Besnoitia*, *Leishmania* and *Trypanosoma cruzi* survive and grow inside macrophages for part, or all, of their stay in the mammalian host.

Extracellular particles which are phagocytised by macrophages enter by invagination of the plasma membrane and appear in phagocytic vesicles³¹. There follows a fusion between the phagocytic vesicle and primary lysosomes, known to contain degradative enzymes, and probably peroxisomes as well, to form secondary lysosomes. In addition, fusion between secondary lysosomes and phagocytic vesicles is also known to occur. At least three different mechanisms can be defined from studies *in vitro* to account for the ability of parasites to evade killing by the macrophage, although the molecular basis for these phenomena and their relevance to the survival of parasites *in vivo* remain unclear.

(1) Failure to fuse: A number of parasites have evolved mechanisms to prevent fusion of macrophage lysosomes with the phagocytic vesicle containing the invader. Human virulent tuberculosis grows in vesicles within macrophages, but these fail to fuse with lysosomes³². Fusion can be induced by opsonisation (coating viable mycobacteria with antibody) but the organism retains its viability³³.

A very similar pattern has been recorded for *Toxoplasma gondii*³⁴. Once again, viable organisms were readily ingested by macrophages *in vitro*, but there was a failure of lysosomes, for example secondary lysosomes labelled with Thorotrast, to fuse with the phagocytic vesicle. Opsonisation of the parasite with antibody permitted fusion of secondary lysosomes with the phagocytic vesicle. In this case, the effect of antibody is to facilitate killing of the *Toxoplasma*. The mechanisms by which

the living parasites are able to block lysosomal fusion in host cells remain largely unknown.

(2) Resistance to killing: As mentioned above, when *M. tuberculosis* is opsonised, lysosomal fusion occurs, but the mycobacteria are perfectly capable of surviving within the lysosome. *M. lepramurium* also grows in vesicles which fuse avidly with primary and secondary lysosomes even without opsonisation³². Similarly, leishmanias grow exclusively in macrophages, and survive and multiply in lysosomes even after fusion has occurred³⁵.

A subtle example of survival in macrophage lysosomes is provided by two species of *Leishmania*. *L. enrietti* produces a disease similar to human cutaneous leishmaniasis or Oriental sore in the guinea pig; *L. tropica* produces a similar disease in some strains of mice. When macrophages from mice sensitised to *L. enrietti* were activated and then challenged *in vitro*, Mauel and Behin³⁶ found them to be resistant to *L. enrietti*. However, when similar procedures were used for activating guinea pig macrophages, *L. enrietti* was not killed. In contrast, activated guinea pig macrophages were highly resistant to *L. tropica*, but failed to resist challenge with *L. enrietti*. Thus, these leishmanias have developed quite specific, but unknown, mechanisms for eluding the destructive capability of macrophages from the species which they normally infect, while being susceptible to the killing mechanisms of activated macrophages from animal species which are resistant to infection by them. An elegant control in these experiments was the simultaneous infection of activated guinea pig macrophages with *L. enrietti* and *Listeria monocytogenes*, to ascertain whether the leishmanias survived by nonspecifically blocking the macrophage cytotoxic mechanisms³⁷. The results obtained were surprising in that, in the same phagosomes, listeria were killed but the leishmanias survived. Thus, there is an extraordinary selectivity in the evolution of resistance to macrophage killing by *Leishmania*.

(3) Escape from the lysosomes: In studies on the interaction of *Trypanosoma cruzi* with normal and activated murine macrophages³⁸⁻⁴⁰ it was observed that within 2 h virtually all trypanosomes were in phagocytic vesicles, and that lysosomal fusion almost invariably occurred (Fig. 2b). Resident unstimulated peritoneal macrophages were as effective as BCG-activated macrophages in killing and degrading a high proportion of the infecting organisms within the first 24 h. However, when infected cultures were examined at 72-96 h, a remarkable picture was seen. Resident macrophages were found to have large numbers of trypanosomes growing within the cytoplasm, and when the Thorotrast labelling technique was used, virtually none of these parasites could be found in a vesicle containing the lysosomal marker (Fig. 2c). They appeared to be able to escape from their precarious state within the lysosome, and take up residence in the cytoplasm, where the macrophage has no specialised mechanism for killing foreign invaders. In contrast, by 3-4 d after infection, macrophages nonspecifically activated by BCG had essentially killed and degraded virtually all of the parasites; only debris was seen (Fig. 2d). Recently, Noguiera and Cohn⁴¹ have been able to activate resident mouse macrophages *in vitro* to kill *T. cruzi* using products of activated lymphocytes.

Jamming the immune response

One of the intuitively obvious adaptive mechanisms by which parasites could elude the normal host response would be by suppressing the immune response itself. Several organisms are known to use this method. For example, immunosuppression has been reported in hamsters infected with *Leishmania donovani*⁴², mice infected with *Toxoplasma gondii*⁴³ and rats infected with roundworm, *Nippostrongylus brasiliensis*⁴⁴. However, by far the most impressive and clinically important instances of the immune suppression of the host response occur in African trypanosomiasis⁴⁵⁻⁴⁷, malaria⁴⁸ and kala-azar. In human sleeping sickness and malaria, elevated levels of IgM are characteristic, on occasion rising to 5 g per 100 ml⁴⁹. In spite of extraordinarily high levels of IgM, 5% or less of these immuno-

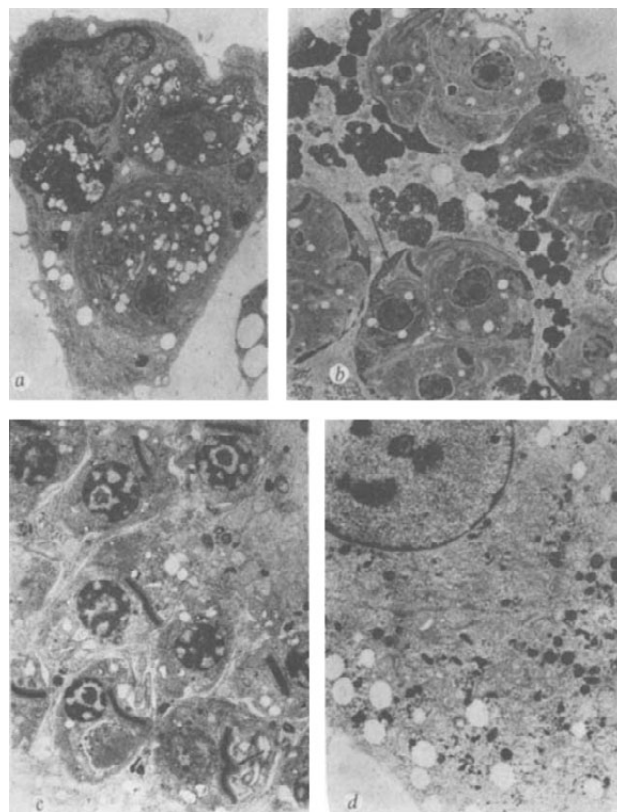


Fig. 2 a, Normal mouse peritoneal macrophages infected with epimastigotes of *T. cruzi* at a ratio of 1:1 after 2 h. Note that parasites are in the process of being digested (arrow). $\times 2,500$. b, BCG-activated macrophage infected with *T. cruzi* at a ratio of 10:1 after 2 h. Thorium dioxide is seen in secondary lysosomes and fusion with parasite-containing vacuole (arrow). $\times 2,500$. c, Normal mouse peritoneal macrophage at a 1:1 infection ratio after 96 h. Trypanosomes are present in cytoplasm where division has begun to occur (arrow). There is no evidence of lysosomal fusion. $\times 4,000$. d, BCG-activated mouse peritoneal macrophage after 192 h of infection with *T. cruzi*. There is no evidence of trypanosomes and some evidence of secondary lysosomes. $\times 2,000$.

globulins have been found to react specifically with antigens of *Plasmodium falciparum* or *Trypanosoma gambiense*, the parasites responsible for malaria and sleeping sickness in West Africa⁴⁹. In both diseases the responses of patients to salmonella antigens and tetanus toxoid, and animals to sheep erythrocytes (SRBC), are markedly reduced⁴⁶. In this paradoxical situation there is a heightened production of antibodies to the parasites with depressed humoral immunity to other antigens.

Several animal models have been developed to probe the mechanisms of this type of immunosuppression. For example, mice infected acutely or chronically with *Plasmodium berghei* malaria show a marked depression in development of antibody producing cells to SRBC in the Jerne plaque assay, the effect being more pronounced on the IgG plaques⁴⁸. Even more striking suppression is seen in mice infected with *Trypanosoma b. brucei*⁴⁷. Perhaps even more impressive than the suppression of antibody production is the marked increase in the background of antibody plaque forming cells to SRBC, generally at least a log higher in infected than in uninfected animals. Thus, infection with plasmodia or trypanosomes causes a marked increase in the background antibody forming cells not only to SRBC but also to donkey, horse or TNP-coupled SRBC. The high background and lack of specificity are strongly reminiscent of the observations made on mouse spleens treated 1-2 d before immunisation with a polyclonal mitogen, such as endotoxin

(lipopolysaccharide, LPS)⁵⁰. There is an increase in immunoglobulin, an increase in polyclonal antibodies which account for the background, and a diminution in specific plaque-forming cells. These experiments suggest that one mechanism for inducing immunosuppression is a polyclonal mitogenic activity of the malaria and trypanosome parasites⁴⁷ causing many clones of B lymphocytes to undergo division and differentiation. Clearly other mechanisms, such as suppression by T cells, may be operative as well⁵¹.

Observations on the immunosuppression by parasites have implications beyond the survival of the specific parasite. It is well known that patients with sleeping sickness have an increased susceptibility to secondary bacterial infections, a problem recognised in some of the earliest studies on the disease. Children in the tropics with acute falciparum malaria also have high rates of bacterial infection; although it must be noted that many also have other parasitic infections, and frequently, protein calorie malnutrition and avitaminosis as well. Nevertheless, antibody responses in children to tetanus toxoid from villages where malaria prophylaxis is carried out are higher than those from comparable villages where malaria infection is frequent⁴⁶. Furthermore it may not be coincidental that Burkitt's lymphoma⁵² occurs mainly in geographical areas coincident with holoendemic malaria. It is not inconceivable that the malaria-induced immunosuppression could provide a favourable environment for development of lymphoma viruses. This possibility has received some experimental confirmation from the studies of Wedderburn in mice in which the incidence of lymphomas in mice infected with both malaria and murine leukaemia virus was dramatically higher than those receiving the virus alone⁵³.

Subversion of the immune system

While the above examples illustrate the ability of parasites to evade the immune system, there are at least two fascinating examples in which the immune system may be requisite for survival of the parasite. *Babesia* and *Theileria* species are protozoan blood parasites transmitted by ticks that can infect mammalian hosts. *Babesia* produces a malaria-like disease in cattle all over the world and *Theileria parva* causes a disease known as East Coast fever which affects cattle primarily in East Africa. *Babesia* Spp. occasionally produce disease in humans.

It has recently been found that *Babesia* seems to require complement activation in order to penetrate the erythrocyte in which it grows. C3 or C5 deficient mice or mice treated with cobra venom are protected from infection⁵⁴.

Unquestionably, the most fascinating case of subversion of the immune system is that of *Theileria* species. These parasites enter lymphocytes and transform them into proliferating lymphoblastoid cells^{55,56}. The intracellular parasite, in its large form called the macroschizont, divides by binary fission, and increases in number 10-fold every 3 d until almost all the lymphoid cells are parasitised⁵⁷. During this process there is a marked lymphocytosis resembling the uncontrolled growth of a leukaemia or lymphoma. From about the tenth day of infection, increasing numbers of the macroschizonts break apart into a large number of microschantons. In the process, host lymphocytes are destroyed and small infectious particles termed micromerozoites are released and invade red cells. There the parasite develops an intra-erythrocytic stage, after which there is a depletion of leukocytes and haematopoietic cells leading to death. In enzootic areas in East Africa, 40% of the calf crop may die from East Coast fever.

The most extraordinary aspect is that when lymphoblasts obtained from cattle infected with *Theileria* are put into tissue culture, they develop into continuously growing lymphoblastoid cell lines with clonal replication of both parasites and cells⁵⁸. Sporozoites from the tick will transform normal bovine lymphocytes *in vitro*, thus *Theileria* species seem to be the only parasite other than oncogenic viruses capable of transforming lymphocytes into continuous growth.

Sceptics may argue that it is unlikely for a protozoan to induce neoplastic transformation of lymphoid cells, and that the parasite may be carrying an oncogenic virus. Yet it has recently been possible to cure the lymphoblastoid cells of the parasite using drugs^{59,60} and they revert to small lymphocytes and stop growing. In any case, the continuous, persistently infected bovine lymphoblastoid cell lines, when transferred back to susceptible cattle, can engender protection to infection with *Theileria parva* or *Theileria annulata* in more than 80% of the instances^{61,62}, and offer the possibility of a vaccine.

Commentary

Several important new programmes in research in parasitic diseases have been initiated since 1976, prompted by increased awareness of the urgent health needs of developing countries and a commitment that basic biomedical research can provide new means for intervening in at least some of these diseases. Among these initiatives are the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, the Great Neglected Diseases Program of the Rockefeller Foundation and the establishment of the International Laboratory for Research in Animal Diseases in Nairobi.

Is there any reason to believe, in light of the subtle and sophisticated ways by which parasites elude immunological attack, that immunological approaches offer any promise? The tremendous advances in understanding basic cellular and humoral immune responses in model systems provide a basis for analysis of the mechanisms of natural and acquired resistance to parasites. The recent development of techniques to produce monoclonal antibodies by means of B-cell-myeloma hybrids (hybridomas) offers the possibility of identifying antigenic determinants, even minor ones, required for engendering resistance to infectious agents. Even if such protective antigens cannot be obtained in large amounts by cultivation of the parasites, as is the case for the blood form of the malaria parasite infectious for red cells or the common, non-varying metacyclic antigen found only on the insect form of the African trypanosomes, the monoclonal antibodies, together with recombinant DNA technology, could be useful for selecting clones of transformed *Escherichia coli* or yeast expressing the protective antigens of parasites which could be used in vaccines. Finally, the recent developments in soluble and liposome adjuvants offer increased possibilities for safe and effective enhancement of immune responses in man.

As in other areas in which basic scientific approaches are applied to disease-related problems, the study of immunity to parasites may provide a new and fundamental knowledge of far wider application than merely to the organisms or disease initially studied. In the early 1940s there was a major effort at the Rockefeller Institute to develop a vaccine against pneumococcal pneumonia. While Avery and his collaborators did not succeed in producing an effective vaccine, they did discover during these studies that DNA was the chemical basis of heredity⁶³. Science too may have its parables.

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articles

Magnetostratigraphy, biostratigraphy and geochronology of Cretaceous–Tertiary boundary sediments, Red Deer Valley

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Integrated magnetostratigraphic and biostratigraphic data for continental Cretaceous–Tertiary boundary sediments in Alberta allow a correlation with recognised sea floor magnetic anomalies 29 and 30. When compared with data from the marine Gubbio section in Italy, the correlation indicates that extinction of the dinosaurs on land was synchronous with foraminiferal extinctions in the sea which are equated with the Cretaceous–Tertiary boundary. We advocate that the base of anomaly 29 be tentatively accepted as the best worldwide physical approximation of the Cretaceous–Tertiary boundary. Eleven K–Ar dates on bentonite sanidines near this boundary yield a mean age of $63.1 \pm 0.5(2\sigma)$ Myr.

THE applicability of geomagnetic polarity reversals to worldwide stratigraphic correlation of sedimentary rocks has become accepted in the past few years. Interest in this application of 'magnetostratigraphy' is shown by the number of recent papers presenting slightly different polarity scales for the Mesozoic and Cainozoic^{1–3}. Limitation of the accuracy of these scales is largely due to the scarcity of good radiometric dates.

The global demarcation of boundaries between major geological time units is very important. The Mesozoic–Cainozoic

boundary has caused a good deal of interest because of the evidence of worldwide extinctions and rapid evolutionary rates at this time. The organisms affected ranged from the largest to some of the smallest of the marine fauna (marine reptiles, ammonites, Foraminifera), and also included the largest continental animals, the dinosaurs, as well as some land flora. The search for causes of such widespread biotic changes has brought to light a large number of plausible explanations⁴. The choice between sudden catastrophic extraterrestrial events (supernovae), and somewhat slower terrestrial processes (global climatic changes), is hampered by the lack of sufficiently accurate and universally applicable chronological data on the contemporaneity of marine and continental extinctions. Correlation of the geomagnetic polarity reversals recorded in sedimentary rocks can provide the necessary accuracy on a worldwide scale. Two notable studies have been reported; one on a marine section near Gubbio, Italy^{5,6}, the other on a continental section in the San Juan Basin of New Mexico, US^{7,8}.

Accurate geochronology of the Cretaceous–Tertiary boundary is difficult because of a lack of explosive volcanicity in those parts of the world which were accumulating a record of fine-grained sedimentary deposits at this time. One of a few places where such volcanicity was prevalent, producing isotopically datable volcanic bentonites and tuffs, is the northwestern US and western Canada. This area was a swampy coastal plain flanking the western shore of a late Cretaceous mid-continental

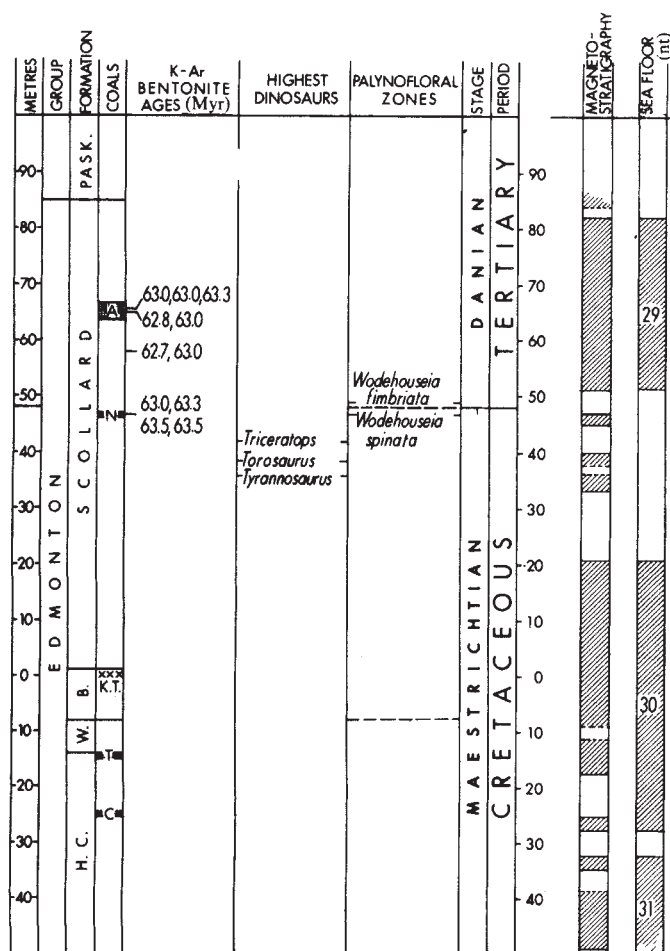


Fig. 1 Biostratigraphy, magnetostratigraphy and geochronology of the upper part of the Edmonton Group, Red Deer Valley, Alberta. B., Battle; W., Whitmud; H. C., Horseshoe Canyon; A., Ardley; N., Nevis; K.T., Kneehills Tuff; T, Thompson; C, Carbon. Cross-hatching denotes normal polarity intervals; dashed boundaries are more tentative than the solid line boundaries.

epieiric seaway with an active volcanic area further to the west. On this fluvial-deltaic lowland lived two of the greatest and the last of the dinosaurs, *Tyrannosaurus* and *Triceratops*.

We report here a study of geomagnetic polarity and K-Ar dating compared with dinosaur occurrences and recently published palynologic studies, carried out on an essentially conformable sequence of mudstones, sandstones, coals and volcanic ashes which encompasses the Cretaceous-Tertiary boundary. One aim of such a study is to obtain reliable dates from a section in which particular sea floor magnetic anomalies can be recognised, to provide data needed for improved temporal calibration of the older part of the marine sea floor anomaly sequence.

Stratigraphy and palaeontology

The Red Deer Valley of south-central Alberta has long been known as a prime collecting area for late Cretaceous dinosaurs⁹; more recently it has been looked at by geochronologists¹⁰ and palynologists¹¹⁻¹³. The 100-m deep valley exposes within a distance of about 50 km the entire thickness (some 350 m) of the largely Maestrichtian Edmonton Group of nearly flat-lying fluvial-deltaic sediments (Fig. 1).

Detailed magnetostratigraphic analysis has been carried out on the upper 135 m of the Edmonton Group, concentrating particularly on the interval near the Cretaceous-Tertiary boundary as defined by a sudden major palynofloral change a few metres above the highest occurrence of dinosaur remains. The palynofloral change includes the abrupt disappearance of all species of *Aquilapollenites* (at least 10) except *A. spinulosus*, as

well as the disappearance of *Wodehouseia spinata*, *Cranwellia striata*, *Proteacidites* sp., and others. They are replaced by such species as *Wodehouseia fimbriata*, *Alnus trina* and *Carpinus subtriangula*¹³. An essentially identical palynofloral change takes place in Montana, Wyoming and the Dakotas at the Hell Creek (Lance)-Fort Union formational contact^{14,15}. In this region the microfloral change also takes place a few metres above the highest occurrence of dinosaur bones, notably *Triceratops*.

In the Red Deer Valley of Alberta the palynomorph break occurs within a 2-m interval centred 1.5 m above a 20 cm bentonite near the top of the Nevis Coal seam. The stratigraphically highest known dinosaur remains still in place in the Red Deer Valley area are part of a scattered skeleton of a large carnivorous dinosaur in Sec. 10, Tp. 34, Rge. 22 West of the 4th Mer. This is the specimen first located by C.M. Sternberg in 1946 and recorded in his field notes as a scattered and broken skeleton of ?*Tyrannosaurus*. He collected part of a skull of a *Triceratops* recorded as occurring 20 feet higher and about 200 yards north of the tyrannosaurid. These specimens have been more recently classified as *cf. Tyrannosaurus rex* and *Triceratops albertensis*, respectively¹³.

The elevation of the *Tyrannosaurus* skeleton was apparently determined by Sternberg's party with an aneroid barometer, and the stratigraphic position determined by fitting this elevation into a more completely exposed section about 1 km to the south. Unfortunately, an error must have been made and the skeletons were inadvertently placed too high (stratigraphically) with respect to the Kneehills Tuff and the Ardley Coal seam. The portion of the tyrannosaurid which is still in place has been relocated and its stratigraphic position redetermined by one of us (J.L.). It lies 10.5 m below the top of the Nevis Coal seam. One assumes that the 20 feet stratigraphic separation between the two skeletons recorded by Sternberg is approximately correct inasmuch as the two sites are very close. The *Triceratops* is thus placed ~4.5 m below the Nevis Coal and 6 m below the Cretaceous-Tertiary palynomorph break. This *Triceratops* skull position is still the highest that we know to have been recorded in Canada.

In the Hell Creek Formation type area in east-central Montana at least three *Triceratops* skeletons have been recovered from an interval reported to be 9-17 m below the top of the Hell Creek Formation¹³. In the same area, two of us (J.L. and H.B.) found unworked dinosaur bones within 4 m of the base of the Z coal which marks the palynofloral change and the Hell Creek-Fort Union contact. The position of the highest dinosaur bones in the type area of the Lance Formation in south-east Wyoming is shown to be about 3 m below the same palynomorph change at a lignite taken to mark the base of the Fort Union Formation¹⁵.

K-Ar geochronology

The portion of the stratigraphic succession near the sudden palynofloral change contains pyroclastic bentonite beds suitable for uncontaminated sampling. Four bentonite horizons (+17.5, +17, +11 and -1 m from the palynofloral change) yielded transparent, unaltered sanidine of the requisite amount and purity for precise K-Ar dating. Duplicate bentonite samples were taken of three of the beds, and the sanidine separates were further split into two grain sizes to check on analytical reproducibility. Heavy liquid and magnetic separation procedures, followed by hand picking, enabled sanidine separates of at least 99.5% purity to be obtained.

Argon was extracted from the samples by flux-fusion *in vacuo*, mixed with pure ³⁸Ar isotope dilutant and purified by hot sponge titanium. Ar isotopic analysis was carried out on a Micro-Mass 6 gas mass spectrometer operating in the dynamic mode. Mass discrimination was determined by measurement of pure air argon in sequence with the Ar determinations in identical analytical conditions. Potassium was determined by isotope dilution using 99+ % enriched ⁴¹K as the tracer. Decay

Table 1 Analytical results from K–Ar dating of bentonite sandine

Sample, elevation (m), mesh size	$^{40}\text{K}^*$ (p.p.m.)	$\frac{^{40}\text{Ar rad}}{^{40}\text{Ar total}} \times 100$	$^{40}\text{Ar}/^{40}\text{K}$	Date* (Myr)
73-1(17.5) +150	10.97	97.6	0.003726	63.0
73-1(17.5) -150	9.68	96.3	0.003723	63.0
75-102(17.5) -80 +120	11.03	96.5	0.003745	63.3
75-100(17) -80 +120	10.82	94.8	0.003740	63.2
75-100(17) -120 +170	9.88	95.7	0.003710	62.8
75-104A(11) -80 +120	11.44	96.1	0.003725	63.0
75-104A(11) -120 +170	11.24	98.2	0.003704	62.7
(0) Palynofloral Cretaceous–Tertiary boundary				
74-1(-1) -80 +120	11.46	97.8	0.003726	63.0
74-1(-1) -120 +170	11.54	97.5	0.003744	63.3
75-400(-1) -80 +120	11.57	96.7	0.003753	63.5
75-400(-1) -120 +170	11.34	96.0	0.003753	63.5

* Constants used: $\lambda_{\beta} = 4.962 \times 10^{-10} \text{ yr}^{-1}$; $\lambda_{\alpha} = 0.581 \times 10^{-10} \text{ yr}^{-1}$; $^{39}\text{K} = 93.2581 \text{ at.}\%$; $^{40}\text{K} = 0.01167 \text{ at.}\%$; $^{41}\text{K} = 6.7302 \text{ at.}\%$.

constants for ^{40}K and natural potassium abundances used in computations are those recommended by Steiger and Jäger¹⁶.

The analytical results are given in Table 1. Using 68 Myr for the age of the base of the Edmonton Group¹⁷, the average rate of sedimentation for the Group is about 65 m per Myr; thus the upper bentonite horizons dated should differ in age from the lower horizon by about 0.25 Myr. This span is within the overall error of the K–Ar determinations, and the dates are averaged to give $63.1 \pm 0.5(2\sigma)$ Myr. Taking into account 2σ errors (precision) in the K-determination ($\pm 0.8\%$), the Ar-determination ($\pm 0.6\%$), the ^{38}Ar -spike volumes ($\pm 0.4\%$) and the decay constants ($\pm 1.7\%$), the K–Ar date for the horizon of palynofloral change in the Red Deer section is 63 ± 2 Myr. Considering indeterminant sources of error, such as sample quality and analytical manipulations, the ± 2 Myr should probably be at least ± 3 Myr.

Careful optical assessment of sample purity showed essentially equal purity for fine- and coarse-grained splits of the sandine samples. Yet the K content (see Table 1) is invariably lower for the fine-grained fraction. The fine-grained fraction may be naturally lower in potassium, but the possibility of undetectable lower-K 'contamination' remains.

Palaeomagnetic data

Approximately 600 samples were collected from some 170 stratigraphic levels (sites) covering 135 m of section. Normally three or four samples were taken at each site, occasionally more. Although the average vertical spacing is thus about 1 m, it varies from 3 m to 10 cm. Closer spacings are associated with polarity boundary levels, and were obtained after examination of preliminary samples. Samples are one-inch cylinders obtained by percussion coring.

The great majority of the remanence measurements were made with a Digico 'complete results' magnetometer, but a Schonstedt SM1 magnetometer was used for a few early measurements. Apart from the Kneehills Tuff and one other volcanic tuff bed, intensities of magnetisation were weak—generally below $1.0 \times 10^{-6} \text{ e.m.u. cm}^{-3}$. Routine alternating-field (a.f.) demagnetisation was carried out using the apparatus as described by McElhinny¹⁸. Completely standardised demagnetisation steps were not used as the material was variable, but peak fields of 75 and 100 Oe were most commonly used. Directional behaviour during progressive magnetic cleaning was highly variable, some specimens being relatively stable and others exhibiting large erratic angular changes. The least stable samples were eliminated by rejecting those associated with $d\theta/dH > 0.5^\circ \text{ Oe}^{-1}$, which reduces the data set to 455. The remaining remanence vectors represent 155 stratigraphic horizons and have been analysed as follows. The virtual geomagnetic pole (VGP) corresponding to each direction was calculated and compared to the well-established Upper Cretaceous–Lower Tertiary palaeomagnetic pole for North America (80–40 Myr, 186°E , 73°N , $N = 11$, $k = 51$, $\alpha_{95} = 6^\circ$ (ref. 19). The angular differences between this pole and each VGP are here called 'polar angles' and are represented in Fig. 2 by the cosine of the polar angle.

Even after rejecting the most unstable material, considerable scatter remains. However, this scatter is not simply random noise—it is evident from Fig. 2 that a stratigraphic zonation exists. We argue that the profile represents a sequence of normal and reversed magnetozones but that secondary magnetic components acquired during the Brunhes epoch mask, to varying degree, the primary remanence of the reversely magnetised strata. An identical situation was recently described by Hillhouse *et al.*²⁰. We adopt a modified version of their procedure

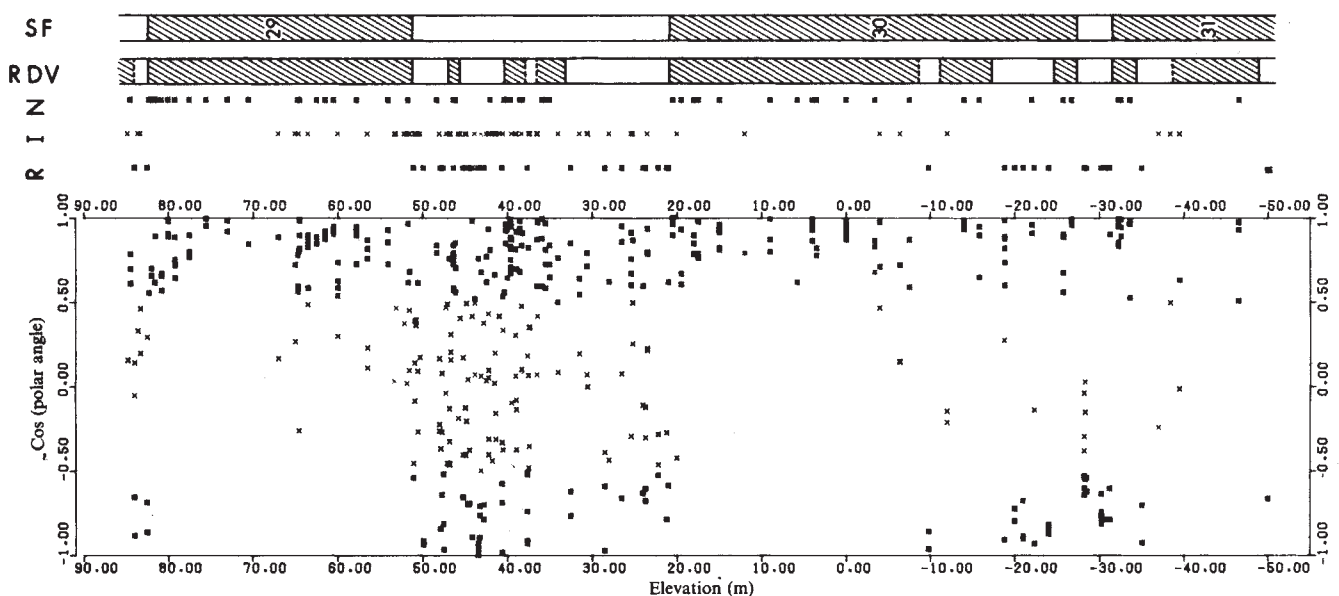


Fig. 2 Magnetostratigraphy of the upper part of the Edmonton Group, and proposed correlation with sea floor magnetic anomalies (Kneehills Tuff is stratigraphic datum as in Fig. 1). See text for geomagnetic polarity assignment procedure. R, reversed; I, indeterminate; N, normal; RDV, Red Deer Valley; SF, sea floor.

for assigning polarity, namely: if all the VGPs at a given site have polar angles less than 60° the site is regarded as normal. If at least one VGP at a site has a polar angle greater than 120° the site is reversed. All other sites are regarded as indeterminate. Hillhouse *et al.* point out that these rules deliberately seek out reversed horizons and this is consistent with the hypothesis that these zones tend to be eliminated by recent magnetic overprinting. Indeed, it is still possible that certain indicated normal sites consist of entirely overprinted material, whereas the same is very unlikely to be true for reversed assignments. The choice of 60° as a cut-off value is arbitrary—we use it because it divides each palaeo-hemisphere in half. This procedure leads to the polarity profile shown in Fig. 2.

Discussion

In Fig. 2 we have attempted to match the Red Deer Valley magnetozones with numbered sea floor anomalies. Recently published polarity scales place the Cretaceous–Tertiary boundary near the base of anomaly 29 (refs 1–3). The fairly long normal polarity zone which embraces the Ardley Coal seam (Fig. 1) thus corresponds to anomaly 29. The other relatively long normal zone (which includes the Kneehills Tuff) is then equivalent to at least part of anomaly 30, and our data suggest that the reversed magnetozone between 29 and 30 contains several short normal intervals.

It is more difficult to identify the short reversed interval which seems to be accepted as a diagnostic marker between anomalies 30 and 31. We have strong indications of at least two comparatively short reversals, either of which would seem to qualify. The lowermost reversal leads to the best relative time span for anomaly 30 versus anomaly 29 according to sea floor data^{1–3}. Our measurements do not go low enough to enable us to use data from strata equivalent to anomaly 31. In the Gubbio marine section, the sampling interval of Roggenthen and Napoleone²¹ allows several short reversals. The scale of the Lowrie and Alvarez⁶ diagrams for the same section makes it difficult to assess their sampling interval, but the reversed zone they have located seems rather high in the section and may correspond to one of our two higher ones.

The presented pattern may still be somewhat oversimplified because we have not placed much weight on several possible short polarity intervals which are represented by more than one site in the unscreened data and are likely to be real, but may represent local geomagnetic 'excursions'.

One or more short normal polarity intervals below anomaly 29 seem to have shown up in other recently published results. Keating *et al.*²² show a short normal in what may be this reversed zone at DSDP site 208. The data of Butler *et al.*⁷ from New Mexico could also be differently interpreted by re-assigning the normal magnetozone they have called 28 to anomaly 29. This would allow correlation of the short normal they have called 29 with our short normal(s) in the reversed zone below 29 (Fig. 3). This re-assignment also removes the palaeontological discrepancies between the New Mexico, Alberta, and Gubbio, Italy sections with respect to the Cretaceous–Tertiary boundary and anomaly 29. Also, because the Cretaceous–Tertiary boundary in the Red Deer Valley is just below the base of anomaly 29 (Fig. 1), this assignment would fit Powell's²³ placement of the boundary in the San Juan Basin in his Kimbeto Member of the Ojo Alamo Sandstone = 'upper conglomerate' of Butler *et al.*⁷. Alternatively, the boundary may well be as low as the unconformity at the base of the conglomeratic member, as suggested by others^{24,25}. Whichever is correct, we agree with Butler *et al.* that the palaeomagnetic evidence suggests the time breaks in the section measured by them must be relatively short.

Considering the rate of sedimentation of the Scollard Formation to be representative of that of the Edmonton Group (about 65 m Myr^{-1}), one average metre represents about 15,000 yr. A check on the validity of this figure may be made using the approach of Kent²⁶. The thickness of the reversed magnetozone between anomalies 29 and 30 in the Scollard Formation is 30 m.

Tarling and Mitchell

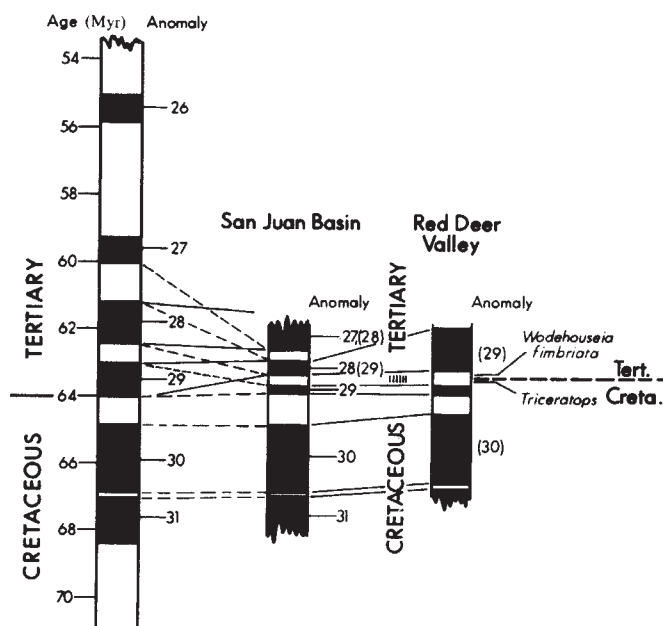


Fig. 3 Suggested correlation of the Red Deer Valley section with the San Juan Basin section and the polarity time scale of Tarling and Mitchell. Dashed tie lines are correlations made by Butler *et al.*⁷. Solid tie lines and anomaly numbers in brackets are correlations suggested in the text. Diagram modified from Butler *et al.*⁷.

This represents a time span of 470,000 yr according to the latest polarity time scale³, providing a calculated sedimentation rate of 63.7 m Myr^{-1} or $15,700 \text{ yr m}^{-1}$. Thus the last dinosaur remains in Alberta were buried about 120,000 yr before the beginning of anomaly 29, and the discrepancy in time between the apparent dinosaur and palynofloral extinctions in this area is about 90,000 yr.

The marine foraminiferal extinctions marking the Cretaceous–Tertiary boundary in the Gubbio section in Italy occur at the base of the *Globigerina eugubina* zone which has been shown to be about 1.5 m below the base of anomaly 29 (ref. 21). Using a mean value of 6.5 Myr for the time span of the Maestrichtian⁵, the sedimentation rate for the Maestrichtian part of the Gubbio section is about 12 m Myr^{-1} . The rate calculated for the reversed magnetozone between anomalies 29 and 30 is 12.8 m Myr^{-1} (ref. 26). Therefore, the time interval between the Cretaceous–Tertiary boundary (based on foraminiferal changes) and the beginning of anomaly 29 was also about 120,000 yr. If our positioning of the base of anomaly 29 in the Red Deer Valley is correct, the foraminiferal Cretaceous–Tertiary boundary and the extinction of the dinosaurs are calculated to be very close in time. Uncertainties in sedimentation rates leave room for some deviation from exact synchronicity. An error in sedimentation rate of as much as 25%, which seems highly unlikely, would produce a maximum difference of about 100,000 yr. Similar studies need to be made on the marine reptilian and ammonite extinctions, but our data indicate approximate synchronicity between at least some marine and continental faunal extinctions at the end of the Cretaceous, by whatever cause.

As the evidence for dinosaur, foraminiferal and floral crises is present just below anomaly 29, it is advocated that the base of anomaly 29 be tentatively accepted as the best global physical approximation of the Cretaceous–Tertiary boundary.

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Observations of seafloor spreading in Afar during the November 1978 fissure eruption

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In November 1978 a telluric crisis was recorded in the Republic of Djibouti, in connection with the opening of new distensive fractures in the Asal graben, the southernmost axis of the seafloor spreading in Afar. A short fissure eruption (7–14 November 1978) developed which allowed interesting observations of ridge-type volcanism and gas composition, and accurate measurement of seafloor spreading in Afar for the first time.

It has been known since the early seventies¹ that the so-called 'Afar triangle' can be divided into two quite different structural units: (1) the Red Sea–Gulf of Aden megastructure (NNW and WNW trends respectively) which belongs to the mid-oceanic ridge type and corresponds to the active boundary between the parting Arabic and African plates, temporarily emerged in Afar; and (2) the northern end of the continental Great Rift Valley of East Africa.

The small Asal graben, approximately 40 km across and 12 km long, located between Asal Lake (155 m below sea-level) and the Gulf of Tadjoura, is the southernmost axis of the present seafloor spreading in Afar² (Figs. 1 and 2). The extremely young age of both volcanic products and tectonic manifestations (faults, uplifts) in the Asal graben³ suggested that a geodimetric network should be set up in that area. The network was positioned by the Institut Géographique National in 1973 over the active axis to measure any increase in distance between the two drifting plates⁴.

The fissure eruption

On 6 November 1978, a series of more than 800 earthquakes was recorded by the Arta seismographic observatory, some of them reaching a magnitude of 3.3 and an intensity high enough to alarm people in the city of Djibouti (70 km away). The actual eruption started the next day and lasted exactly one week.

A set of two dozen parallel normal faults was observed in a belt more than 1500 m wide in the axial area. Some of the faults reached 10 km long. The general fault direction was 300–320° (NNW), that is, parallel to the Red Sea tectonic trend. No significant lateral shifting was detected. The spacing between fault lips varied from a few tenths of a millimetre to more than a metre. The maximal throw observed was more than 0.5 m. The

whole set constituted a graben the depth and width of which can easily be determined by new geodimetric measurements. The increase in distance between the African and Arabian plates, roughly the sum of each fault width, can be determined exactly.

This event supports the hypothesis that the seafloor spreading mechanism works by sudden jerks, occurring a few times per century, rather than by a continuous smooth distension⁵. Average rates of seafloor spreading in the Asal rift area⁶ should be referred to in terms of 1.5 m per century rather than 1.5 cm per yr.

The fissure eruption developed over a 0.75-km long segment of one of the normal faults, directly on the active axis. We called it 'Ardoukoba' (slope down, in Afar language) after the place name⁶. It was exclusively effusive and no actual explosions occurred: the only comparatively violent gas exhausting phenomena were lava fountains, which were generally 20–100 m above lava level. Lava flows spread rapidly over the depression. A lava pool developed, together with the building

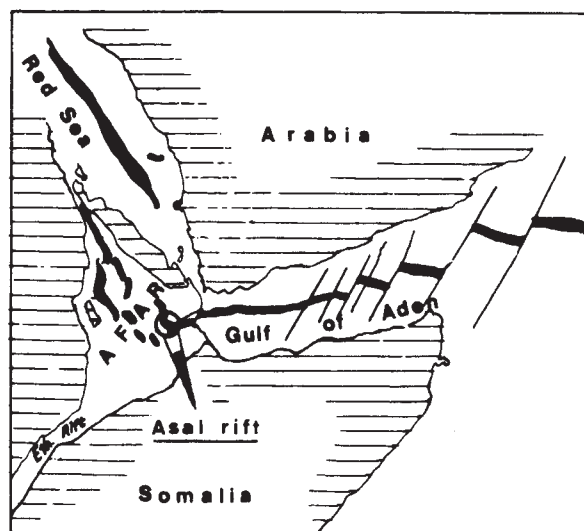


Fig. 1 Location of the Asal rift in the Afar depression. Black areas are zones of generation of new oceanic crust.

Table 1 Composition of the Ardoukoba gas samples (mol %)

	% Total H ₂ O	N ₂	O ₂ + Ar	CO ₂	SO ₂ (mol % of the 'dry' gases)	H ₂ S	CO	H ₂	COS	C/S	CO/CO ₂	p _{O₂} (atm)
Precision		±1%	±1%	±1%	±1%	±1%	±10%	±1%	±10%			
A92	62 ± 3	45.0	11.0	8.3	35.3	2 × 10 ⁻⁴	0.15	0.11	10 ⁻⁴	0.24	1.8 × 10 ⁻²	10 ^{-9.43}
A89	59 ± 7	50.9	12.8	6.8	29.3	6 × 10 ⁻³	0.11	0.02		0.24	1.6 × 10 ⁻²	10 ^{-9.33}
									to			
A88	ND	47.4	11.2	6.6	34.7	0	0.09	0.02		0.19	1.3 × 10 ⁻²	10 ^{-9.15}
G82	47 ± 7	57.9	15.4	3.8	22.6	3 × 10 ⁻³	0.16	0.06	5 × 10 ⁻⁴	0.16	4.2 × 10 ⁻²	10 ^{-10.15}

Analysed by gas chromatography in helium with a catharometer cell. H₂ was detected in argon and H₂S using a flame ionisation detector. H₂O was determined by weighing. Measurements were from flask samples from flowing lava at 1,070 °C. ND₁ not determined.

up of a steep spatter rampart, 40 m high, erected on the fracture's lips as an oblong crater.

For the first few days of the eruption, the boiling lava pool had a nearly 2,000 m² surface, which was continuously overturned by the fountaining. The violence of the phenomenon prevented us from reaching the crater lip to sample eruptive gases and we had to wait for the last phase of the eruption before we could do so. A plume, the velocity of which was estimated at 10 m s⁻¹, escaped from this crater. From 12 November the lava pool surface was restricted to 400 m². The eruption ended on 14 November with a short, more explosive phase. The total gas output for the duration of the eruption (six effective days) is evaluated as 6 × 10⁹ m³.

Sampling

Gas samples were collected on the fracture on 13 November from lavas flowing out of the crater (temperature = 1,070 °C). Severe conditions restricted numbers of sampling to four only. More numerous (but less representative) samples were collected on 14 November from cracks in recent cooling lava flows. Gases were collected in evacuated glass flasks, containing either an alkaline solution or P₂O₅ dessicator, connected to a 1.5-m long silica pipe. The alkaline-type flask showed the gas to be considerably mixed with air. Therefore, we also used a technique which allows the gas sample to remain in the same dilution as the plume, preserving the gaseous ratios. It was analysed with a high sensitivity (<1 p.p.m. for most gases investigated): the previously dehydrated gases are inspired by a peristaltic pump, stored in a Teflon bag and then immediately analysed by specific reactive 'Dräger' tubes.

Temperature measurements were taken simultaneously using a chromel–alumel thermocouple (±1 °C). Locations, temperatures and gas compositions are reported in Tables 1 and 2.

Results

Air was admixed with the magmatic gas found between the flowing lava and the upper solidified crust through which we sampled. Atmospheric values for N₂/O₂ + Ar ratios, calculated from the O₂ + Ar contents in the four glass flasks, assuming that all Ar comes from air contamination, yield an excess nitrogen which either corresponds to a true magmatic excess or reflects a partial oxidation of the gas by atmospheric oxygen.

The gases were found to be richer in sulphur than carbon. The C/S atomic ratio (0.2) is lower than that typical of high temperature basaltic gases sampled from central vents (1.5 ± 0.3)⁷. Otherwise, similar C/S ratios have been found in gases sampled from early degassed basalts in Hawaii⁸ and particularly from comparable lava flows from Mt Etna^{9,10} or the Surtsey eruption (1966–67 samples)¹¹. This lower ratio may be typical of a later degassing stage of the lava. Conversely the 14 November gas samples (evacuated flask samples and Teflon bag samples), collected from cooling lavas at sub-solidus temperatures are richer in CO₂ than in SO₂, a feature more characteristic of gases exsolved from solidifying hot rocks. In these samples H₂S is also rich relative to SO₂.

CO/CO₂ ratios are in the range 1.3–4.2 × 10⁻². Experimental data¹² showed that: (1) the oxidation state of a high-temperature gas phase is consistent with the oxygen fugacity in the melted basalt; (2) the CO/CO₂ ratio is the most reliable redox ratio, compared to H₂/H₂O or H₂S/SO₂, and is more sensitive to oxidation and to secondary gains or losses caused by condensations, reactions or additions.

Under a pressure of 1 atm and collection temperature (1,343 K) conditions, the oxygen partial pressure in equilibrium with CO and CO₂ can be calculated from:

$$\log p_{O_2} = 2 \left(\frac{\Delta G_f^\circ}{9.150T} - \log \frac{p_{CO}}{p_{CO_2}} \right)$$

where $\Delta G_f^\circ = -135,330 + 41.74T$ (calculated from standard thermodynamic data¹³).

Table 1 lists the four p_{O₂} values obtained, and the mean oxygen partial pressure of the gas is found to be p_{O₂} = 10^{-9.40} atm. This is in remarkable agreement with the p_{O₂} value in the Erta Ale gases (Afar)¹², and consistent with the oxygen fugacities measured or deduced from mineral assemblages in basalts^{14,15}.

Water vapour is generally the major gas present and often amounts to 80–90 mol % of the total, even in higher quality samples collected previously¹². The interesting point in the present results is the relative deficiency in H₂O content of the Ardoukoba samples. The measured H₂/H₂O ratio (about 10⁻³) is not consistent with the mean p_{O₂} calculated above: a p_{O₂} of 10^{-9.4} atm would lead to an equilibrium H₂/H₂O ratio of about 10⁻². As water vapour clearly condensed inside the flasks during

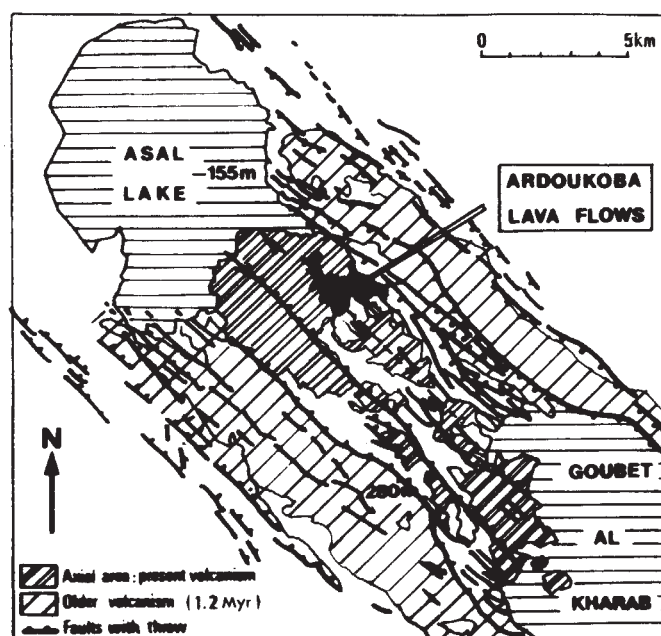


Fig. 2 Position of the Ardoukoba lava flows in the Asal rift.

Table 2 Composition of gas samples and sublimates from cooling lavas

Precision	CO ₂ (p.p.m.) ±5–10%	SO ₂ (p.p.m.) ±5–10%	H ₂ S (p.p.m.) ±10–15%	HCl (p.p.m.) ±10–15%	C/S	Cl/S	Sublimates (wt %)
W lava flow	2,000	230	5	22	8.5	0.09	SO ₄ ²⁻ 63.07
W lava flow	4,200	850	12	30	4.9	0.03	
W lava flow	4,000	600	11	24	6.5	0.04	
W lava flow	1,100	100	3	9	10.7	0.09	Na 24.66
W lava flow	1,500	120	6	15	11.9	0.12	
W lava flow	400	20	0	0	17.4	—	K 5.83
W lava flow	600	50	1	4	11.8	0.08	
S spatter	1,200	160	3	9	7.4	0.05	Ca 0.48
E lava flow	800	80	2	9	9.7	0.10	
W base cone	1,400	160	5	10	8.5	0.06	Fe 0.61
Crater rim	2,000	320	6	3	6.0	0.01	Cu 5.35
							Cl Mg B <0.1

Sublimates were analysed using an EDS. (Five analyses of green and white sublimates.) Measurements were from Teflon bag samples (810–910 °C).

sampling (temperature at the opened end of the sampling pipe reached more than 400 °C), we can be certain that no water vapour was lost through the flask's stopcock. We suggest two hypotheses to explain this discrepancy: (1) A partial oxidation of the initial H₂ occurs (1 mol % calculated from H₂/H₂O = 10⁻²), consistent with the partial deficiency in the O₂ contents when air contamination is calculated on the N₂ basis. (2) A preferential enrichment in H₂O occurs by continuous adsorption on the dessicator in excess during the few seconds the flasks were open. If this were so, the equilibrium H₂O contents might have been about five times less than they were actually found to be in the samples.

At present it is not possible to decide between these two hypothetical mechanisms, and they may have acted together. Also, the H₂O content of the samples has to be considered as an upper limit to the water vapour fraction of these eruptive gases which therefore appear particularly anhydrous.

The HCl content in samples from cooling lavas (Table 2) is surprisingly low as it has been shown from many volcanoes that similar gases from early degassed lavas are generally more chloride-rich than those from central vents. Atomic Cl/S ratios in Ardoukoba gases are still lower than Cl/S ratios in gases collected from the Erta Ale lava lake (0.17) or from lava flows of the oceanic Surtsey eruption (0.03–0.5)¹¹.

From the proximity of highly saline waters in Asal Lake (NaCl > 350 g l⁻¹), 2 km away, and of seawater in the Ghoubet, 10 km away (Fig. 2), one could have expected a marine type

contamination of the magma and gases, the eruption point of which was situated about 100 m below sea level.

In fact, the collected samples appear rather lacking in vapour and chloride poor. Moreover, microprobe energy dispersion spectrometer (EDS) analyses of incrustation deposits from around the fuming points (Table 2) show no anomalous sodium enrichment and show also that these sublimates are particularly depleted in chloride, boron and magnesium.

The total lavas welled out by the eruption amounts to 16 × 10⁶ m³, the average thickness of the 1.6 km² lava field being estimated at about 10 m. This highly fluid lava contains a high proportion of phenocrysts, mainly large crystals of plagioclase (bytownite) up to 4 cm long, and far less olivine and clinopyroxene. It is identical to the particularly plagioclase-rich basalts widely outcropping in the active part of the Asal graben^{5,16} (Table 3).

Discussion

Note that this short, but volumetrically important, eruption is similar to numerous recent ones, the evidence of which we observed in this area and throughout the whole northern and central Afar depression, and is probably representative of most of the fissure eruptions in rift areas. It is interesting as a reference for the so many, but invisible, submarine fissure eruptions. In particular, it is useful to compare the ratio of the massic gas and lava outputs with the volatile amounts trapped in fresh sub-oceanic basalts. 16 × 10⁶ m³ of solidified basalt with a density of 2.7 g cm⁻³ yields 43 × 10⁶ tons of lava discharged by the Ardoukoba eruption, and the mass of 6 × 10⁹ m³ of emitted gas, the density of which could be considered to be ~0.25 g l⁻¹ at 1 atm and 1,100 °C, is 1.5 × 10⁶ tons. Then, the mass gas output amounts for 3% of the lava production. This is rather small, when compared with the recently measured gas discharge from other volcanoes¹⁷, which widely exceeds the initial volatile content the corresponding erupted magma could supply.

This leads us to consider that the magmatic reservoir from which the Ardoukoba eruption developed may have been a closed system. The relative 3% massic gas to lava output is scarcely a few times the volatile content in sub-oceanic ridge basalts¹⁸.

This low gaseous output, the particularly low water vapour content, the apparent lack of marine contamination which might have been expected, the short duration of the eruption and the highly crystallised state of the produced lava, make us think that the magma intrusion which supplied the Ardoukoba eruption was quite narrow and probably close to the surface, and hence reduced the disturbance the eruption could have brought about in the geological surroundings.

Table 3 Chemical composition (wt % oxides) of the Ardoukoba lava and phenocrysts

	Lava flow	Pyroclastite	Olivine	Pyroxene	Plagioclase
SiO ₂	48.01	48.01	37.55	51.22	46.55
TiO ₂	1.21	1.09	—	0.2	—
Al ₂ O ₃	20.72	20.43	—	1.4	33.91
FeO + Fe ₂ O ₃ *	8.90	8.59	—	—	—
FeO	—	—	15.36	4.26	0.21
MnO	<0.1	<0.1	0.13	—	—
MgO	3.50	3.99	46.82	17.91	—
CaO	15.26	15.46	0.14	22.44	17.90
Na ₂ O	2.35	2.29	—	—	1.43
K ₂ O	<0.05	<0.05	—	—	—
P ₂ P ₅	<0.1	<0.1	—	—	—
Cr ₂ O ₃	—	—	—	0.33	—
Total	100.2	100.1	100	97.8	100

Analyses were carried out using an energy dispersion spectrometer

* Based on equimolar ratios.

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Fluorine in Iceland and Reykjanes Ridge basalts

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Unlike chlorine and bromine, fluorine is not appreciably outgassed from basalts erupted above 500 m depth along the Reykjanes Ridge and subaerially over Iceland. The mantle beneath Iceland seems to be twice as rich in fluorine as the asthenosphere source of normal ridge basalts south of 61°N.

OCEANS and atmosphere are generally accepted to have formed over geological time by the outgassing of volatiles from the Earth's interior during volcanism¹. But very little is known about the abundance, distribution and relative fractionation of volatiles in the mantle. Nor do we fully understand the nature of the processes of transport and release of volatiles to the Earth's surface. The study of volatiles in lavas derived from the mantle is complicated by the tendency of such elements to be partly lost during the ascent of magmas, either before erupting at shallow depth under the sea or during subaerial eruption. Furthermore, volcanic gases collected near volcanic vents may not be juvenile, but partly or totally derived from fluids of meteoric origin. Thus, knowledge of the geochemical evolution of volatiles in the mantle has lagged behind that of the more refractory elements. The study of submarine basalts and basaltic glasses, pioneered by Moore², has opened up new opportunities for the investigation of volatiles.

Unni and Schilling³ have described the distribution of chlorine and bromine in basalts from the Reykjanes Ridge and Iceland, contrasting subaerial with submarine volcanism as affected by hydrostatic pressure. They found that both chlorine and bromine were drastically fluxed out from lavas erupted at depths of less than 500 m below sea level (approximately 50 bar hydrostatic pressure), coincident with the depth of extensive vesiculation of water observed in several instances^{2,4–8}. Chlorine and bromine degassing occurs although neither of the two halogens is saturated in the silicate melt in the pressure–temperature conditions of eruption³. Fluxing of sulphur and selenium has also been observed at this depth^{7,8}. Unni and Schilling³ have considered the possibility of fluxing of chlorine and bromine as due to a strong tendency for these two volatiles to separate into the aqueous fluid phase relative to the melt during vesiculation, in accordance with the experimental partition data of Kilinc and Burnham⁹. However, the amount of water actually exsolving seemed to be insufficient, and CO₂ (and/or SO₂) was invoked as an additional possible major flux carrier for outgassing chlorine and bromine.

Fluorine, although another member of the chemically related halogen group, might be expected to behave differently in a silicate melt–aqueous fluid system. Its smaller ionic radius, 1.34 Å compared with 1.81 Å for chlorine and 1.96 Å for bromine, permits fluorine to substitute easily for OH[−] in hydrous minerals or melts¹⁰. The few experimental data available for partitioning of fluorine and chlorine between silicate melts and a co-existing H₂O fluid phase at high temperature and pressure^{9,11} suggest partition coefficients of 3:1 for fluorine in favour of the melt, but less than 1:30 for chlorine. The existing data are for granitic-type melts. As far as we know there is no corresponding data for melts of tholeiitic composition.

We report here the fluorine content of basalts from the Reykjanes Ridge–Iceland profile previously analysed by Unni and Schilling³ for chlorine and bromine. This part of the Mid-Atlantic Ridge, as it shoals from 1,380 m to its subaerial expression in the south-west neo-volcanic zone of Iceland, affords an excellent opportunity to study the behaviour of volatile elements as a function of hydrostatic pressure. Locations and depths from which the samples were dredged are shown in Fig. 1. This area also spans from rare earth¹² and isotope data^{13,14} two apparently separate and distinct mantle sources of magma with a zone of mixing between them. Furthermore, it has been shown³ that chlorine and bromine variations along the Reykjanes Ridge–Iceland profile fit quite well a predictive model compounded from a curve of the distribution of large ion lithophilic trace elements (LILE) from two different adjacent mantle sources and their mixing, and a curve representing the degassing of a volatile from lavas derived from a single homogeneous mantle source as a function of decreasing hydrostatic pressure above 500 m of depth. The abundance of fluorine in the same basalt samples should not only reveal the distribution of fluorine along the Reykjanes Ridge and its extension beneath Iceland, but also yield useful information relevant to degassing mechanisms of the three halogens and their relative fractionation during degassing.

The fluorine profile

Fluorine was determined by a specific ion electrode procedure adapted from the method of Ingram¹⁵ for fluoride in silicate rocks, combined with the procedure of Huang and Johns¹⁶ for fusion and preparation of sample solutions. The method was standardised against the literature values of Flanagan¹⁷ for the rock standards AGV-1 and JB-1, and was reproducible to ±15 p.p.m. in the relevant concentration range. The samples are fresh basalts dredged from the axis of the ridge¹², and analyses

were carried out on powders prepared for the most part from the variolitic zone of pillows, and a few from glasses from pillow rims. A summary of individual fluorine concentrations in pillow basalts from the Reykjanes Ridge and Iceland is given in Table 1, where they are compared with the chlorine values of Unni and Schilling³ determined on the same samples.

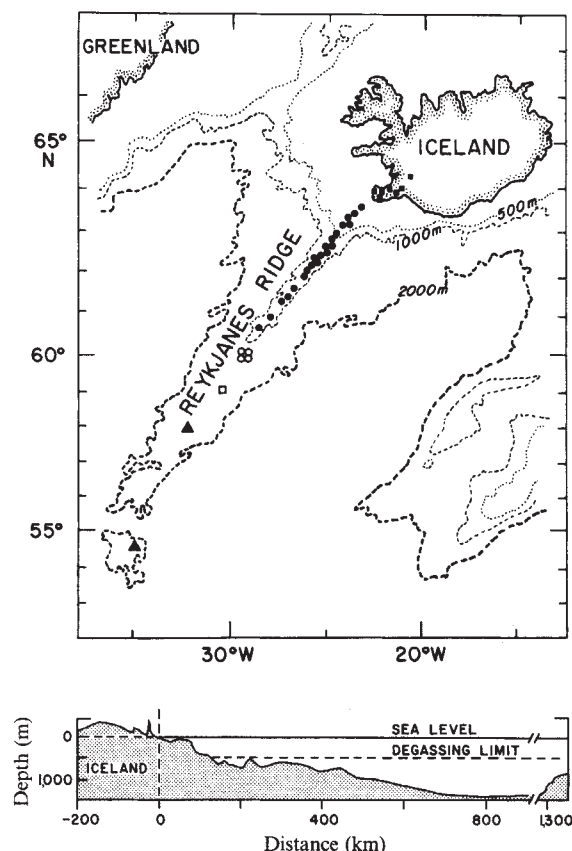


Fig. 1 The Reykjanes Ridge and Iceland, showing depth contours and sample locations. Cruise numbers are: ●, TR 101; ○, TR 41; □, GLJ; and △, TR 100; and subaerial samples are designated by ■.

The variation of fluorine content with latitude (Fig. 2) shows a progressive increase of fluorine towards Iceland. The fluorine profile resembles those of refractory incompatible trace element ratios such as $[La/Sm]_{EF}$ (Fig. 2a), or of isotope ratios^{13,14} of $^{87}Sr/^{86}Sr$ or $^{206}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$. Furthermore, fluorine variation does not seem to be related in any simple way to Al, Mg, or Ca variation in these basalts along the Reykjanes Ridge–Iceland profile. Nor is it related to silica content, which remains constant at $50 \pm 0.3\%$ in submarine samples and drops to 47–49% over Iceland (data not shown). Indices of major element fractionation such as $\Sigma Fe/\Sigma Fe + Mg$ and Na_2O/CaO pass through a maximum over the Iceland shelf coincidental with the en echelon displacement of the ridge axis^{18,19}, whereas fluorine continues increasing towards Iceland. These observations rule out the possibility of explaining the fluorine increase towards Iceland as being due to increasing fractional crystallisation or decreasing degrees of partial melting of a single mantle source toward Iceland (see for example refs 12, 20–22 for discussions on the subject).

In marked contrast to the other volatiles Cl, Br, S and Se, fluorine does not show any appreciable degassing above the apparent 500 m critical depth of extensive vesiculation of water vapour observed in several instances^{2,3,5,7,8} (Fig. 2). Although fluorine follows the same trend as chlorine up to 63°N at depths

greater than 500 m, the fluorine content of basalts in the northernmost section of the profile remains high while chlorine decreases due to progressive degassing with decreasing depth of eruption³. The failure of fluorine to degas is further emphasised in Fig. 3, which compares variation of fluorine with variation in lanthanum, a refractory element; potassium, a less refractory element; and chlorine, a volatile element. For both lanthanum versus fluorine and potassium versus fluorine the Iceland field (subaerial) overlaps with the trend for the Reykjanes Ridge. But in the case of chlorine versus fluorine, the Icelandic field has moved down along the chlorine axis and is quite distinct from the Reykjanes Ridge basalts, because of the loss of chlorine during subaerial eruption³.

The relative fractionation of fluorine with respect to chlorine in the Reykjanes Ridge basalts is shown in Fig. 2, using Cl/F ratios from Table 1. The ratio increases on approaching land, apparently due to enrichment of the larger ion in the Iceland mantle source compared with the LILE-depleted asthenosphere. But at depths less than 500 m and in subaerial samples the ratio drops drastically because of the loss of chlorine with concurrent water vesiculation. From a maximum value of 1:1 the Cl/F ratio drops to about 0.1, or by approximately an order of magnitude.

Table 1 Fluorine content of Iceland and Reykjanes Ridge basalts (pillow interiors)*

Station	Lat (N)	Long (W)	Distance† (km)	Depth (m)	F (p.p.m.)	Cl‡ (p.p.m.)	Cl/F
Iceland							
IC-17	64°47.2'	20°42.7'	–139	+400	248	45	0.18
IC-11	64°16.75'	21°07'	–64	+150	211	24	0.11
IC-36	64°12.6'	20°01.2'	–59	+250	155	30	0.19
IC-57	63°55.8'	22°26.5'	–29	+72	270	99	0.37
IC-62	63°58.7'	21°58'	–23	+140	193	60	0.31
IC-47	63°58'	21°44.5'	–22	+460	214	64	0.30
IC-58	63°54.8'	22°26'	–14	+70	248	93	0.38
TR 101							
15D-13A	63°34.7'	23°42.2'	33	33	233	128	0.55
18D-1	63°28.1'	23°51'	49	43	251	172	0.68
11D-2	63°16.3'	24°12.4'	78	75	230	173	0.75
12D-7	63°12.9'	24°16'	86	258	270	199	0.73
14D-5C	63°11.2'	24°27.6'	91	345	261	232	0.89
6D-9	62°59.7'	24°41.9'	118	400	222	170	0.76
3D-1A	62°47.5'	25°09.6'	147	620	179	152	0.85
35D-3A	62°42'	25°12.3'	160	580	176	185	1.05
2D-1,2	62°37.4'	25°26'	172	633	217	226	1.09
1D-2	62°35.6'	25°27.5'	176	618	206	211	1.02
22D-1	62°22.4'	25°50.7'	208	715	153	166	1.08
23D-1	62°21.3'	25°36.8'	210	655	169	116	0.70
34D-6	62°16.1'	26°08.4'	221	500	151	96	0.64
24D-6C	62°05'	26°17.8'	248	692	182	51	0.28
27D-1	61°44'	26°52.9'	298	600	120	140	1.17
29D-5A	61°05.9'	27°52.7'	390	810	86	85	0.99
30D-10A	61°05.9'	27°54.2'	390	792	82	65	0.79
31D-9C	60°44'	28°54.2'	440	710	86	16	0.19
33D-6A,B	60°27.4'	28°53.1'	482	923	137	36	0.26
TR 41							
D46-1	61°22'	27°24'	352	650	124	67	0.54
D22-1	60°01.2'	29°28.5'	543	978	126	64	0.51
D20-3	60°01.2'	29°20.8'	543	948	254	108	0.42
D18-2	59°59.7'	29°32.5'	545	1040	99	62	0.63
D38-2	59°59.1'	29°31.5'	547	925	132	71	0.54
GLJ-10	58°52.1'	30°56.7'	705	1383	98	70	0.71
TR 100							
26D-10	57°41'	32°34'	885	1380	180	69	0.43
23D-10	54°15'	35°24'	1340	880	127	28	0.22

* Our precision for F analyses of standards AGV-1 and JB-1 were $439 \pm 19(1\sigma)$ and $365 \pm 9(1\sigma)$, respectively. Corresponding recommended values are 435 ± 20 and 360 (ref. 17).

† Distance SW along the Reykjanes Ridge Axis; taken equal to zero at the SW tip of the Reykjanes Peninsula.

‡ Cl values from Unni (ref. 46).

Yet, despite the evidence suggesting absence of degassing of fluorine from the Reykjanes Ridge basalts, fluorine is often found to be present in volcanic gases and emanations.

Measurements of the F/Cl ratio in gases from Hawaiian volcanoes range from 0.3²³ to 0.003²⁴. Moreover the incidence of fluorine poisoning in grazing livestock following a major Icelandic eruption has been directly correlated with wind direction, distance from the eruption vent, and thickness of tephra layers²⁵. Thorarinnsson²⁶ describes measurements of 350 p.p.m. fluorine in ash particles less than 0.06 mm in diameter compared with 50 p.p.m. in an 0.25 mm fraction, such enhancement has been interpreted²⁷ as indicating adsorption of gaseous HF on the surface of the ash during its eruption. Thorarinnsson²⁶ has estimated that at least 3×10^4 tons of fluorine were discharged with the tephra of the Mt Hekla eruption in 1970.

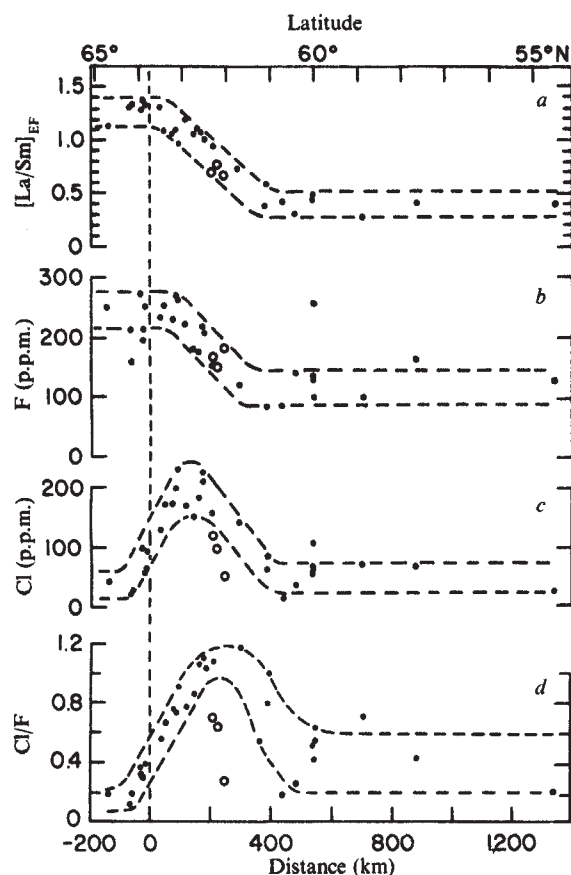


Fig. 2 Comparison of the F profile (b) with the La/Sm enrichment factor ratio (a), Cl (c), and the Cl/F ratio (d). ○ are anomalously low in Cl (and also Br) but not in F or refractory elements. Morphologically, they are rounded vesicular basalts (not pillows), and represent probably lava erupted subaerially, subsequently eroded by wave action during subsidence (or submergence). The low Cl and Br, but not F nor LILE contents, seem to corroborate our observations made previously on ship.

Our evidence for the lack of any substantial fluorine degassing at eruption depths of less than 500 m along the Reykjanes Ridge would seem paradoxical. This is not the case. A simple calculation using the Rayleigh distillation equation²⁸ predicts that, if a maximum of 0.5–1.0% H₂O vapour exsolved, the chlorine and fluorine concentrations of the vapour would be of the order of 5,000 p.p.m. and 70 p.p.m. (assuming vapour–melt partition coefficients of 30 and 0.33) respectively. The F/Cl ratio in the vapour would be 0.014, thus well within the range observed on land^{23,24}. The fluorine content of the remaining magma would have increased only by 1 p.p.m. maximum, which is well within the analytical uncertainties.

The lack of appreciable outgassing of fluorine along the ridge may to some extent be further tested by comparing variation in fluorine with variation in a refractory lithophilic element which might vary coherently with fluorine. Data from the area of the Mid-Atlantic Ridge encompassing the Azores platform, a section well below the 500 m critical depth of degassing, indicate that fluorine and strontium, or fluorine and phosphorous do not fractionate appreciably relative to each other during partial melting or fractional crystallisation^{29,30}. The two ratios remain constant despite clear evidence from ⁸⁷Sr/⁸⁶Sr data^{31,32} of a change in mantle source beneath the Azores. Figure 4 shows variation of the F/Sr and F/P ratios with distance along the Reykjanes Ridge–Iceland profile.

Equations for least-squares best-fit slopes are:

$$F/P = 3.28 \times 10^{-5} d + 0.289$$

$$F/Sr = 1.22 \times 10^{-4} d + 1.75$$

where d , the distance in km south-west along the Reykjanes Ridge axis, is set equal to zero at the south-west tip of the Reykjanes Peninsula on Iceland. In other words, both ratios remain essentially constant along the profile. The subaerial data are insufficient to establish any significant loss of fluorine. Even if the sample IC-17 (anomalously high in fluorine content) is not considered, the small drop in F/Sr and F/P ratios over Iceland would suggest a maximum loss of only 5–10%. We conclude from this and from the Cl/F ratio profile that unlike chlorine and bromine, fluorine does not appreciably degas from lava erupted subaerially or above the 500 m depth. A comparison of the maximum and minimum Cl/F ratios would indicate a relative

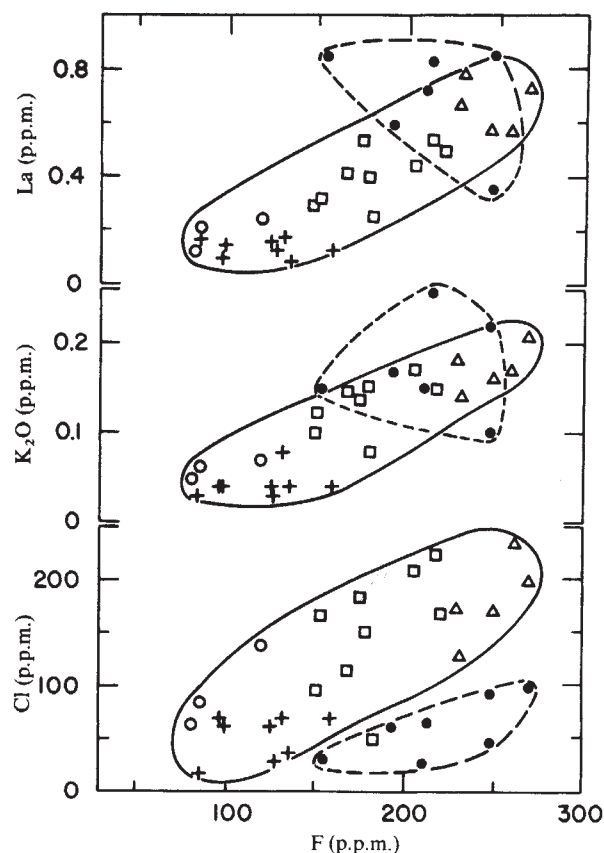


Fig. 3 Comparison of F variation with La (refractory), K (less refractory), and Cl (volatile). —, Reykjanes Ridge submarine samples; ---, Icelandic subaerial field. ●, 63–64° N; □, 62–63° N; ○, 61–62° N; +, S of 61°.

fractionation of these two elements of at least a factor of 10 in the shallow water and subaerial basalts.

Yet, the Rayleigh distillation calculation previously discussed predicts only a 25% drop in the Cl/F ratio due to vesiculation of a maximum of 0.5–1% water vapour. The Cl/F ratio could be further reduced to 33% of the maximum observed, if up to 50% of the melt underwent shallow depth fractional crystallisation at the same time as vesiculation occurred. A fluorine bulk crystal–melt partition coefficient of 0.02 was used in the calculation³⁰. Note also that these calculations are based on fluid–melt partition coefficients for granitic melts^{9,11}. Unless in the case of basaltic melts such vapour–melt partition coefficients are significantly reduced for fluorine and increased for chlorine, vesiculation of a major gas phase other than H₂O, such as CO₂, would have to be invoked to account for the major Cl/F drop at less than 500 m depths, as suggested by Unni and Schilling³ for chlorine alone. A corollary of the chlorine outgassing mechanism proposed for lava erupted above 500 m depth or subaerially is that emanated volcanic gas counterparts should have a Cl/F greater than unity. This seems to be corroborated by the Cl/F ratios of volcanic gases, which though quite variable, are usually greater than unity, and often greater than 10 (refs 23, 24, 33, 34). The preferential affinity of fluorine for the melt compared with chlorine might suggest a different structural model for the solubility of fluorine as opposed to chlorine in silicate melts, although Burnham's³⁵ solubility model would not support this.

Fluorine content of the two mantle sources

As previously inferred, the fluorine distribution in the Reykjanes Ridge–Iceland basalts is consistent with Schilling's model¹² of two mantle sources for this region, one depleted in LILE and one more enriched, with an intermediate zone of mixing between them. The fluorine content in the section between 54° and 61°N (deep sea) averages 114 ± 24 p.p.m. (1σ), which may be taken as characteristic of mid-oceanic basalts derived from a LILE-depleted asthenospheric source. North of 63°N and including seven Icelandic tholeiites from the south-western neo-volcanic zone, fluorine averages 232 ± 32 p.p.m. (1σ), with more scatter in subaerial than submarine samples. Fluorine between 61° and 63°N grades between the two levels in a manner grossly consistent with the mixing of two sources. These results suggest a 2–3 fold relative enrichment of fluorine in the mantle source of basalts from the Iceland platform over the source of the ridge south of 61°N.

An estimate of the fluorine content of the two mantle sources of this region may be made by assuming: (1) partial melting of 25–30% over the entire area to produce the magmas, and (2) a reasonable partition coefficient for fluorine between solid and melt during partial melting. Estimates of a fluorine partition coefficient can be made in two ways, considering that no experimental values are available. First, fluorine varies nearly coherently with strontium in the processes of partial melting and fractional crystallisation^{29,30}. The strontium partition coefficient between basaltic groundmass and a number of minerals present as phenocrysts measured by Philpotts and Schnetzler³⁶ varies greatly, depending on the nature of the phenocrysts. Assuming that the two mantle sources under consideration were originally composed of a lherzolite containing 60% olivine, 20% orthopyroxene and 20% clinopyroxene, the bulk partition coefficient for strontium (and hence also F) between such an assemblage and the melt (D_{Sr}) would be about 0.04 ($D_F = D_{Sr} = 0.60 \times 0.01 + 0.20 \times 0.1 + 0.20 \times 0.06$).

Second, although the mineral phases of the mantle are speculative, judging from the fluorine value determined by Huang and Johns¹⁶ for the geochemical peridotite standard PCC-1, a fluorine value of 13 p.p.m. would be anticipated for a residual peridotite which has been depleted in LILE by partial melting. Since D is defined as C_R/C_L , where C_R is the concentration in the solid residue after partial melting and C_L the concentration

in the melt, then, assuming 13 p.p.m. for C_R and a minimum of 100 p.p.m. F for C_L in the melt, a partition coefficient (D_F) of a maximum of 0.13 would be obtained, which is not far from the partition coefficient for fluorine calculated from a lherzolite mantle model using strontium as an analogue.

The use of a reasonable partition coefficient of 0.06 for fluorine would suggest a fluorine content of 74 ± 10 p.p.m. for the Iceland mantle source, and 36 ± 9 for the LILE-depleted asthenosphere, assuming a degree of melting of 25–30% and using the partial melting equation of Schilling and Winchester³⁷.

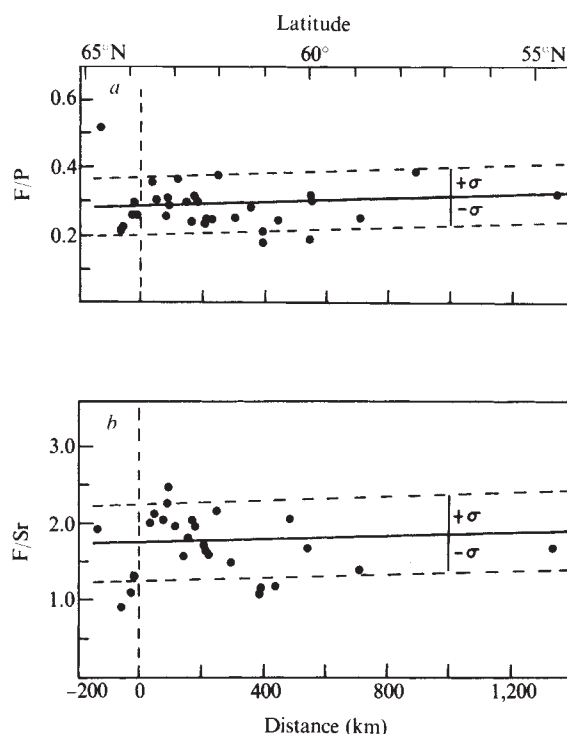


Fig. 4 Variation of F relative to a, P; and b, Sr along the Reykjanes Ridge–Iceland profile. See text for equation of line by linear regression.

Fluorine in basaltic glasses

We have also examined the possibility of fluoride exchange with seawater, either during submarine basalt formation at the time of quenching, during subsequent cooling of the pillow lava, during low temperature weathering, or during hydrothermal circulation. For this purpose the fluorine content of fresh glasses, glasses altered to various degrees, and a palagonite were compared with pillow interiors (mostly sampled within the variolitic zones). The samples were either from the same basalt pillow or from other pillow fragments recovered from the same station.

Table 2 summarises our results for fluorine in 15 glasses and a sample of palagonite removed from the glassy rim of a pillow basalt from dredge haul TR101-27D, and compares these concentrations with fluorine values of pillow interiors from Table 1. Five of the 15 glasses matched rock fluorine within analytical error and two more were only slightly higher. The others were enhanced in fluorine by a factor of 1.3–3.0, and the enrichment is not apparently related to depth of eruption, latitude or the quantity of fluorine in the corresponding crystalline samples. In no case was fluorine in glass significantly lower than in the more crystalline pillow interior. Several glass samples from one station showed highly variable enrichment in fluorine

content (TR101-27D). A similar increase in content in quenched glassy rims over more crystalline interiors has been observed for several volatiles other than fluorine, such as sulphur³⁸, ⁴⁰Ar (ref. 39), and H₂O (ref. 5).

Table 2 Fluorine content in Reykjanes Ridge basalts (glass rims)

Station	Distance* (km)	Depth (m)	F_{glass} (p.p.m.)	$F_{\text{rock}}^{\dagger}$ (p.p.m.)	F_g/F_r
TR 101					
11D-2G	78	75	458	230	1.99
11D-s.g. [‡]			344	(230) [§]	1.49
35D-2g	160	580	172	176	0.97
35D-3g			236	(176)	1.34
1D-10g	176	618	230	(206)	1.12
22D-2g	208	715	154	(153)	1.01
27D-1g	298	600	172	120	1.43
27D-13g			364	(120)	3.03
27D-glass			160	(120)	1.33
27D-palagonite			436	(120)	3.63
30D-2g	390	793	202	(82)	2.46
30D-12g			232	(82)	2.83
TR 41					
D22-s.g.	543	978	142	(126)	1.16
D20-s.g.	543	948	251	(254)	0.99
D18-s.g.	545	1040	93	(99)	0.94
D36-s.g.	547	925	138	(132)	1.05
Average					$1.54 \pm 0.70(1\sigma)$

Samples were collected with RV Trident during cruise TR 41 and TR 101.

* See Table 1.

† Pillow interior (mostly variolitic zone).

‡ s.g., Station glass (loose glass found in the dredge haul).

§ Rock values in parentheses are from a different pillow within the same station.

The cause of fluorine enhancement in some of these glasses is uncertain. There are several possibilities: (1) Incorporation of fluorine from seawater either during quenching or later by low-temperature weathering, would seem unlikely. The fluorine content of seawater is only 1.3 p.p.m. (ref. 40) while the content in pillow interiors is 82–270 p.p.m. Equilibrium of the glass with seawater would tend to reduce fluorine in the glass, if the partition coefficient previously discussed were to apply. (2) Entrapment of bubbles rising from the melt, as proposed by Moore and Schilling⁷ for sulphur enhancement in glasses, might be considered. However, in the case of fluorine this explanation also seems unlikely, as first, we have shown that fluorine is not appreciably degassed from vesiculating lava and hence is not mobilised during vesiculation (see the previous calculation on distillation of fluorine), and second, the observed fluorine enhancement was not related to hydrostatic pressure. (3) Fluorine could have been lost from pillow interiors, rather than enriched in the glassy rims. The mechanism of Corliss⁴¹, by which incompatible elements are concentrated in residual interstitial liquid in a crystallising melt—to be subsequently removed by hydrothermal waters circulating through micro-cracks and fissures, is supported by finding fluorine as one of the elements enhanced in interstitial melts from the Alae Lava Lake of Hawaii⁴². Fluorine data on hydrothermal solutions such as found in the Galapagos Region⁴³ could be a test of this possibility, when available. Clearly, more information is needed to resolve the question of high glass/rock ratios for fluorine.

Conclusions

Fluorine does not outgas like chlorine and bromine from submarine lavas erupted at a depth of less than 500 m, the depth of rapidly increasing vesiculation. While fluxing by vesicle-forming major volatiles, such as H₂O and/or CO₂, seems to explain the outgassing of chlorine and bromine at shallow depth, such a mechanism is ineffective in removing appreciable quantities of fluorine. Our observations of outgassing of halogens and relative fractionation of chlorine and fluorine during intense vesiculation

at depth shallower than 500 m are also consistent with experimental partition data for the relative distribution of fluorine and chlorine between silicate melts and a co-existing aqueous phase. The proposed outgassing model is also consistent with the contrasting Cl/F ratios less than unity in outgassed lavas and greater than unity in volcanic gas counterparts usually reported. The contrasting outgassing behaviour of fluorine and chlorine (and bromine) emphasises the need of establishing whether a distinct structural model for the solubility of fluorine in silicate melts as compared with chlorine and bromine is required.

The distribution trend of fluorine in basalts from the Reykjanes Ridge and Iceland profile is consistent with the hypothesis of two mantle sources and their mixing for the origin of basalts of this area. The mantle source of the Iceland basalts seems to be enriched by approximately a factor of two over the source of the ridge basalts erupted south of 61°N.

An explanation for our finding of fluorine enrichment in some glass samples over more crystalline pillow interiors (variolitic zone) will require further information.

O'Hara⁴⁴ has considered extensive and long-lived contamination of Icelandic magma chambers by previously erupted basalts which may have been hydrothermally altered by circulating seawater. We cannot comment on this mechanism from our fluorine data which does not include hydrothermally altered basalts. However, we note that the ²⁰⁷Pb/²⁰⁴Pb versus ²⁰⁶Pb/²⁰⁴Pb trend previously observed for the Iceland-Reykjanes Ridge profile¹⁴ does not point towards estimated lead isotope ratios for seawater⁴⁵, as would be expected if the O'Hara model were to be applicable to this region.

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t-Haplotypes of the mouse may involve a change in intercalary DNA

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The naturally occurring t-haplotypes of the mouse exhibit a set of peculiar genetic properties, including strong suppression of crossing over in the segment of chromosome 17 between the loci of T and H-2. Study of the genetics of mutant haplotypes suggests that the observed effects on meiosis and embryonic development may be due to an altered form of intercalary DNA (iDNA) in the relevant chromosomal region (band 17B).

THE t-haplotypes of the mouse¹⁻⁴ occur commonly in the wild and thus must be considered part of the usual mouse genome. Furthermore, they are located near to the major histocompatibility complex (H-2 complex) on chromosome 17, thereby inviting speculation concerning any possible evolutionary or other genetic significance of this close association^{5,6}. Their properties include homozygous lethality and various effects when heterozygous, including enhancement of the effect of the mutant gene for brachyury, T, sterility or segregation distortion in males, and strong crossover suppression in chromosome 17.

The nature of the genetic change in t-haplotypes remains unknown. They are thought to involve some kind of chromosomal changes, but there is no evidence for major structural rearrangements such as inversions⁷, and no indication that rDNA is involved⁸. We suggest here that t-haplotypes involve a change in moderately repetitive DNA (iDNA) intercalated between the structural genes in the segment of chromosome 17 occupied by the haplotypes, extending approximately from the locus of brachyury, T, to the H-2 complex (Fig. 1). This suggestion arises from a study of the retention, loss or alteration of the various properties in 'mutant' t-haplotypes which have been derived from naturally occurring ones by crossing over or other means, and we regard it as a development of our earlier suggestions that t-haplotypes involved 'heterochromatin' (ref. 9) or changes in interstitial heterochromatin¹⁰.

Heterozygotes for brachyury T typically have short tails, but t-haplotypes interact with T, so that T/t heterozygotes are tailless. When homozygous, naturally occurring t's are lethal or semi-lethal during embryogeny, and fall into six lethal complementation groups, with partial complementation between groups in terms of viability¹. Surviving t^x/t^y males (where t^x and t^y are from different groups), and also males homozygous for semi-lethals, are sterile. Furthermore, males heterozygous for a single t-haplotype, that is, +/t or T/t, although fertile, transmit t to the offspring with an abnormal frequency, usually having a large excess of t-carrying young. In addition, natural t-haplotypes strongly suppress crossing over in both sexes in the segment of chromosome 17 extending from T to H-2.

A further characteristic is that of mutating to other forms, with loss or occasionally gain of one or more of the other properties. This 'mutation' is usually, but not always, accompanied by crossing over within the region of strong crossover suppression. Correlation of the genetic properties of the various mutant haplotypes with the apparent position at which the crossover occurred has made it possible to map factors underlying the various properties. The most extensively studied batch of

mutant haplotypes is derived from the haplotype t⁶ (Fig. 1), which belongs to the t⁰ complementation group⁹⁻¹³. Less detailed results with mutants from other haplotypes suggest that they may be broadly similar to t⁶ (refs 14-16).

Lethal t-haplotypes are usually maintained in balanced lethal stocks, using crosses of T/t/t^x × T/t/t^x, both T/T and t^x/t^x being lethal. In such stocks mutant t's are occasionally found which have lost the lethality and are viable when homozygous. In all cases this loss of lethality is accompanied by crossing over between T and tf. This suggested the existence of a lethal factor (the LS-factor), located near the locus of tf, and lost whenever the wild-type allele of this locus is lost (Fig. 1, for example, haplotypes t^{h2} and t^{h3}). This was confirmed by the finding in linkage tests of the complementary type of crossover (t^{h17} and t^{h18}, Fig. 1)^{9,11}. Mutant t's of this kind are lethal either when homozygous or in compound with t⁶, but they do not produce taillessness when heterozygous with T; thus, T/t^{h17} and T/t^{h18} are short-tailed like T/+. This leads to the interpretation that the T-interaction effect is due to a factor located near the locus of brachyury. Further study of the two complementary types of mutants revealed that all the viable mutants were male fertile, and t^{h17} and t^{h18}, which retained the lethality, also retained the male sterility of t⁶. It thus seems that a factor for male sterility is close to that for lethality¹⁰. For simplicity, the two factors are shown as one in Fig. 1 (the LS-factor), but it is possible that they are separable.

The next factor to consider is segregation distortion in males. Mutant viable haplotypes are of two main kinds (Fig. 1): those with normal ratio (t^{h3}) and those with a low transmission ratio from males (t^{h2}). This suggests that a factor for low ratio might be located between T and tf. This concept was confirmed by the discovery of the mutant low¹⁷, later renamed t^{low} (ref. 10), which arose by crossing over in a stock carrying t^{h17}. It has the property of causing a low transmission (<50%) from males of the chromosome on which it lies, but it is viable when homozygous, and does not modify the effect of T. t^{low} is thus regarded as a middle-piece of t-chromatin carrying the abnormal ratio factor, designated the A-factor, but not the T- or LS-factors. The finding (mentioned below) of further t^{low}-type mutants in the present work confirms this interpretation. In males carrying t^{low} and another t^x with an abnormal transmission ratio, either high or low, the two haplotypes t^{low} and t^x are transmitted with equal frequencies. Thus, the first requirement for abnormal transmission is that the A-factor should be present and heterozygous. However, the A-factor alone gives a low ratio. The high ratio seen in naturally occurring haplotypes seems to depend on other parts of the t-chromatin. Thus, it has been suggested that the spaces between the T-, A- and LS-factors are occupied by abnormal t-chromatin of some kind, and that the high transmission ratio of natural t-haplotypes is due to the presence of the A-factor and interaction of this factor with all parts of the t-chromatin^{9,10}.

A further property of natural haplotypes so far unexplained is suppression of recombination over the segment of chromosome 17 from T to H-2. Mutant haplotypes which arise by recombination between T and tf usually permit recombination in this region, although still with some suppression. Such weak suppression could be due to partial loss of the crossover-

suppressing property over the whole T-H-2 segment, or mutant haplotypes might still suppress crossing over strongly over their own (reduced) length and allow free recombination elsewhere. If the latter explanation were correct, double heterozygotes for two complementary and overlapping mutant haplotypes (for

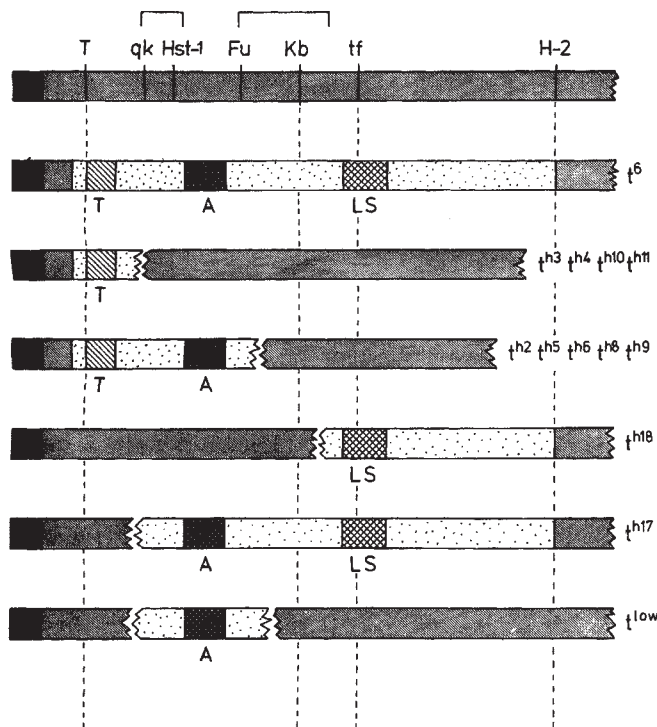


Fig. 1 Diagrammatic representation of the structure of the t^6 -haplotype, of various mutant haplotypes derived from it, and of the corresponding normal segment of mouse chromosome 17. The centromere is depicted to the left in black. Some relevant known loci in the normal chromosome are brachyury, T; quaking, qk; hybrid sterility, Hst-1; fused, Fu; knobbly, Kb; tufted, tf; and H-2. In the t-chromatin the postulated T-int-, A- and LS-factors are hatched and the intervening stretches of altered chromatin are stippled.

example, t^{h2} and t^{h17} , Fig. 1) should exhibit strong crossover suppression, even though each haplotype separately permits recombination. Conversely, in double heterozygotes for non-overlapping haplotypes (for example, t^{h2} and t^{h18} , Fig. 1), crossing over should occur freely in the segment of normal chromatin between the two stretches of t-chromatin. We have already presented evidence that in the double heterozygotes for t^{h18} and various viable T-end mutants, such as t^{h2} , t^{h6} and t^{h11} , considerable crossing over in the relevant segment can occur⁹.

To test the overlapping haplotypes, animals heterozygous for t^{h2} and t^{h17} were bred. These particular haplotypes were known to overlap as both included the A-factor. The recombination between T and tf in these doubly heterozygous animals was then compared with the value in animals carrying t^{h17} only, and therefore having normal chromatin over part of the relevant distance (Table 1). In animals carrying only t^{h17} , the T-tf recombination is considerably enhanced (the normal value is about 8%), apparently an effect of the translocation T(1; 17)190Ca with which t^{h17} is inextricably associated. In the double heterozygotes t^{h2}/t^{h17} , however, recombination was strongly suppressed and was less than one-tenth of that in single t^{h17} heterozygotes (1.5 ± 0.6 compared with $18.1 \pm 0.9\%$, Table 1). The interpretation was that both t^{h2} and t^{h17} suppressed crossing over strongly over their own length, and thus, as the two haplotypes overlapped, recombination was strongly suppressed over the whole distance from T to tf.

Other evidence

Hammerberg and Klein¹⁸ showed that the crossover-suppressing effects of t-haplotypes extended beyond tf, approximately to the H-2 complex but little, if at all, beyond this. Thus, it is assumed that t-chromatin extends to H-2, but the absence of mutant haplotypes with breaks between tf and H-2 prevents a similar study of the basis of the crossover suppression in this segment. However, both t^{h17} and t^{h18} retain the H-2 haplotype of t^6 , from which they were derived, even though they had been crossed to other stocks carrying other H-2 genes for many generations before their H-2 genotype was tested¹². This is consistent with the idea that each t-haplotype suppresses crossing over for its own length, and that t^{h17} and t^{h18} extend distally as far as H-2, but it is not critical evidence.

There is little evidence concerning crossover suppression by mutants derived from natural haplotypes other than t^6 . Bennett¹⁹ bred animals doubly heterozygous for t^{38} , a viable mutant with low transmission ratio derived from t^0 (ref. 15), and t^{low} . As t^{38} has a low ratio it must have included the A-factor (thus resembling t^{h2} in Fig. 1) and must have overlapped t^{low} . Bennett found no recombination between t^{38} and t^{low} , suggesting that t^{38} , like the t^6 -mutants, suppresses crossing over throughout

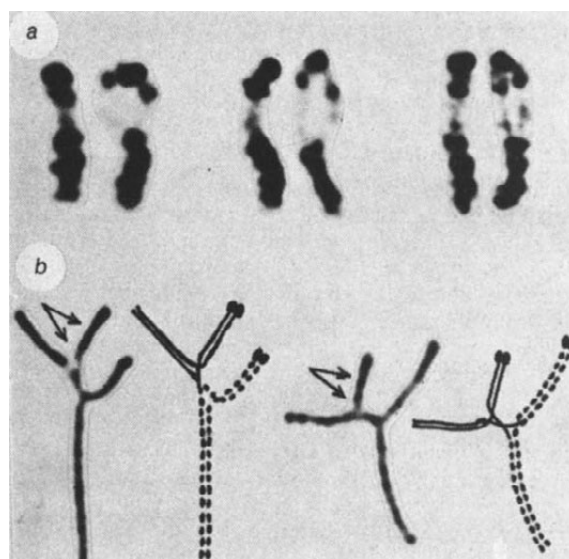


Fig. 2 a, Giemsa-stained chromosome 17 bivalents at pachytene. Examples of the synchronous and asynchronous synapsis observed in the large, pale 17B band in, left to right, +/+, T/+ and +/t⁶ animals. Note also that in +/t⁶ there is no detectable heterozygosity in the bivalent in length or staining intensity. b, Synaptonemal complexes and the diagrammatic interpretation of quadrivalents they form in T/+ and +/t^{w5} T138 heterozygotes. Chromosome 17 and its translocated segments are shown as the continuous line, chromosome 9 and its segments as the broken line. Both quadrivalents show equivalent synapsis in the region of the 17B band (arrowed). The synaptonemal complexes were freed and fixed using the method described by Tres²⁴ but were then sedimented on to glass slides and stained with aqueous silver nitrate before viewing with the light microscope.

its own length. Pizarro and Dunn²⁰ showed that t^{w35} , a viable mutant derived from t^{w32} , decreased recombination in the T-tf interval and increased it in the tf-H-2 interval. This is consistent with the suggestion that t^{w35} suppressed crossing over strongly over its own length, leading to a compensatory increase in recombination in the adjacent segment.

Table 1 The effect of overlapping t-haplotypes on T-tf recombination

Parents	Sex of heterozygote	Offspring				Total	Recombination (% $\pm$ s.e.)
		T+	Ttf	++	+tf		
T(t^{h17})+/+tf $\times$ +tf/+tf	♀	399	79	84	352	914	17.8 $\pm$ 1.3
	♂	327	86	72	377	862	18.3 $\pm$ 1.3
T(t^{h17})+/ t^{h2} tf $\times$ +tf/+tf	♀	70	—	2	81	153	1.3 $\pm$ 0.9
	♂	93	2	3	79	177	2.8 $\pm$ 1.2
		T++	T+tf	T t^{h2} +	T t^{h2} tf	Total	
+(t^{h17})+/ t^{h2} tf $\times$ Ttf/+tf	♀	21	—	—	17	38	
	♂	44	—	—	41	85	
Combined data (t^{h17})+/ t^{h2} tf	♀				RC	Total	
	♂				2	191	1.0 $\pm$ 0.7
					5	262	1.9 $\pm$ 0.8

Animals were classified for tail length within a few days (usually 1 day) of birth and for tufted, tf, at about 4 weeks. Any doubtful animals were kept and genetically tested. For the third group of crosses only the T-carrying offspring are shown as the remainder provide no linkage information. The seven recombinant animals in the crosses involving t^{h17}/t^{h2} were all genetically tested and shown to carry recombinant haplotypes. Both Ttf animals proved to be T(t^{low})tf/+tf. The 5++ animals all gave tailless offspring when crossed to T/+ (that is, they carried the T-int factor). Two permitted recombination between T and tf, and were shown to be due to chiasmata proximal to and distal to the overlap region; the remaining three are still being tested. RC, recombinant offspring.

Thus, the available evidence, though not conclusive, is entirely consistent with the suggestion that in any t-haplotype, whether natural or a derived mutant consisting of proximal, central or distal region, crossover suppression is co-extensive with the t-chromatin.

Chiasma formation

In principle, crossover-suppression observed among the progeny may be due either to failure of chiasma formation or to normal chiasma formation with elimination of the products of crossing over.

Because at meiosis the chromosome 17 bivalent cannot be unequivocally identified, this question has been studied in t-haplotypes by marking the chromosome with translocations. In mouse translocation heterozygotes, if there is a chiasma in each arm of the multivalent then a ring is observed at meiotic metaphase I. If one arm lacks a chiasma then a chain configuration is formed, and if two arms lack chiasmata there may be either a chain-of-three plus univalent, or two unequal bivalents. Forejt²¹ studied the configurations formed at first meiotic division in males heterozygous for T(9;17)138Ca, T(1;17)190Ca and T(8;17)43H. In each case animals carrying t^{12} had a lower mean number of chiasmata in the translocated chromosomes than did their T/+ siblings. The reductions were 0.27, 0.25 and 0.45 chiasmata per multivalent in T138, T190 and T43, corresponding to map distances of 14, 13 and 23, respectively. Thus, they were approximately of the right order, as the recombination in T138 heterozygotes is reduced by about 9% when the haplotype t^6 is present²².

Because of the importance of this point, we have repeated Forejt's work, using T138 and three other t-haplotypes, t^6 , t^{w5}

and t^{w32} . Meiotic configurations in males heterozygous for T138Ca and a t-haplotype were compared with those in littermates or other close relatives heterozygous for T138 and T/+ (Table 2). In each case the number of chiasmata per multivalent was significantly lower in males carrying t^6 , t^{w5} or t^{w32} than in their T/+ relatives. For t^6 and t^{w5} the reductions were 0.23 and 0.21 chiasmata per multivalent, respectively, quite close to the value found by Forejt for t^{12} . With t^{w32} the difference was greater, about 0.39 chiasmata, but as only two t^{w32} males were studied, little importance can be attached to this point.

Our main conclusion is that, as a reduction in number of chiasmata in T138 multivalents is produced by four different t-haplotypes, t^6 , t^{12} , t^{w5} and t^{w32} , then the crossover suppression caused by t is due to reduced chiasma formation, rather than elimination of products of chiasmata.

In other organisms factors which lead to chiasma suppression have been classified as asynaptic or desynaptic, according to whether synapsis fails or whether synapsis occurs normally but chiasmata do not form²³. It would be valuable to know whether t-haplotypes should be considered as asynaptic or desynaptic. We attempted to study chromosome pairing in chromosome 17 of males with or without t by light microscopy of pachytene preparations. These studies revealed that the large pale Giemsa-staining band 17B pairs asynchronously even in normal (+/+ or T/+) animals and may be found unpaired when more proximal and distal bands are closely apposed (Fig. 2a). This is relevant as t-chromatin is thought to be located in this band (see below). However, there was no difference in pairing between +/ t^6 or +/ t^{w5} and T/+ animals, either in these Giemsa-stained chromosomes or in synaptonemal complexes observed by light microscopy, using T138 as a marker of chromosome 17 (Fig. 2b). Thus, chiasma suppression in t-haplotypes is probably of

Table 2 Multivalent configurations and chiasmata at first meiotic division in male mice heterozygous for T138Ca and various t-haplotypes

Haplotype	No. of males	RIV	No. of cells with configuration			Total	No. of chiasmata	Difference
			ChIV	ChIII+I	20II			
t^{w5}	3	734	221	3	397	1,355	3.25	
Ttf	5	1,353	571	8	320	2,252	3.46	0.21
t^{w32}	2	453	161	3	414	1,031	3.04	
Ttf	4	1,244	291	9	400	1,944	3.43	0.39
t^6	7	2,176	570	22	1,189	3,957	3.24	
Ttf	5	1,860	452	16	503	2,831	3.47	0.23

Males were bred by crossing Ttf/t+ $\times$ T138/T138. Testis preparations were made by the method of Evans *et al.*³⁴ and stained with toluidine blue. Observations were made of cells in diakinesis-metaphase I using coded slides. RIV, ring of 4; ChIV, chain of 4; ChIII+I, chain of 3+univalent; 20II, no multivalent configuration.

the desynaptic type, but further work, probably involving electron microscopy, is needed to confirm this point.

The chiasma suppression of t-haplotypes is typically studied in heterozygotes, having normal chromatin on the homologous chromosome. It would be valuable to know whether chiasmata are also suppressed when t-chromatin is present on both homologues. Does the abnormality involve mutual recognition of t-chromatin and normal chromatin, or is the low chiasma-forming property of t-chromatin autonomous?

Crosses intended to test this point are in progress but have been inconclusive. Similarly, the crossover haplotypes derived from the t^{h2}/t^{h17} heterozygotes are being studied to determine whether any have resulted from chiasmata in the overlap region. To date, two t^{low} type mutants have been found (which give no information on chiasma position) and one each of mutants resulting from chiasmata proximal to and distal to the overlap region. It seems likely that chiasmata are suppressed at least to some extent, when t-chromatin is homozygous as well as when it is heterozygous, but further work is needed on this point.

Location and extent of altered chromatin

Genetically, the crossover-suppressing effect of t-haplotypes extends from the T to H-2 loci, but apparently not far beyond

H-2 (ref. 18). There is little evidence about the effect proximal to the T-locus.

Cytogenetically, part (and perhaps all) of the t-chromatin lies in band 17B, the large band in chromosome 17, which stains lightly with Giemsa. The evidence for this comes from the relative location of translocation breaks and genetic markers (Fig. 3a). Translocations T(1; 17)190Ca and T(16; 17)43H have breaks in band 17B (refs 25, 26), and T(9; 17)138Ca in band 17D (ref. 25) (Fig. 3b). Genetically, the T138 break is 3cM distal to H-2 (ref. 27) and that in T43H is very close to H-2K (ref. 28). Thus, it seems probable that the H-2 complex, and hence the distal end of t-chromatin, lies near the distal end of band 17B. The locus of *tf* (near the centre of the t-chromatin) apparently lies distal to the T190 break, and hence also in 17B (ref. 29). The position of the brachyury locus is less certain. Bennett¹ reported that the brachyury allele, T^{bp} , which involves a deletion extending from T to qk, was cytogenetically detectable as a shortening of the A2 band. However, we have been unable to confirm this. In preparations made from 12-day embryos, some T^{bp}/t^{h2} and others $+/t^{h2}$, no consistent differences between the pairs of chromosome 17 were detected (Fig. 4). Hence, it is not clear whether the T locus lies in band 17A2, or whether it too lies in 17B. Thus, the stretch of altered chromatin in t-haplotypes probably occupies the whole of band 17B and may extend proximally into the 17A bands.

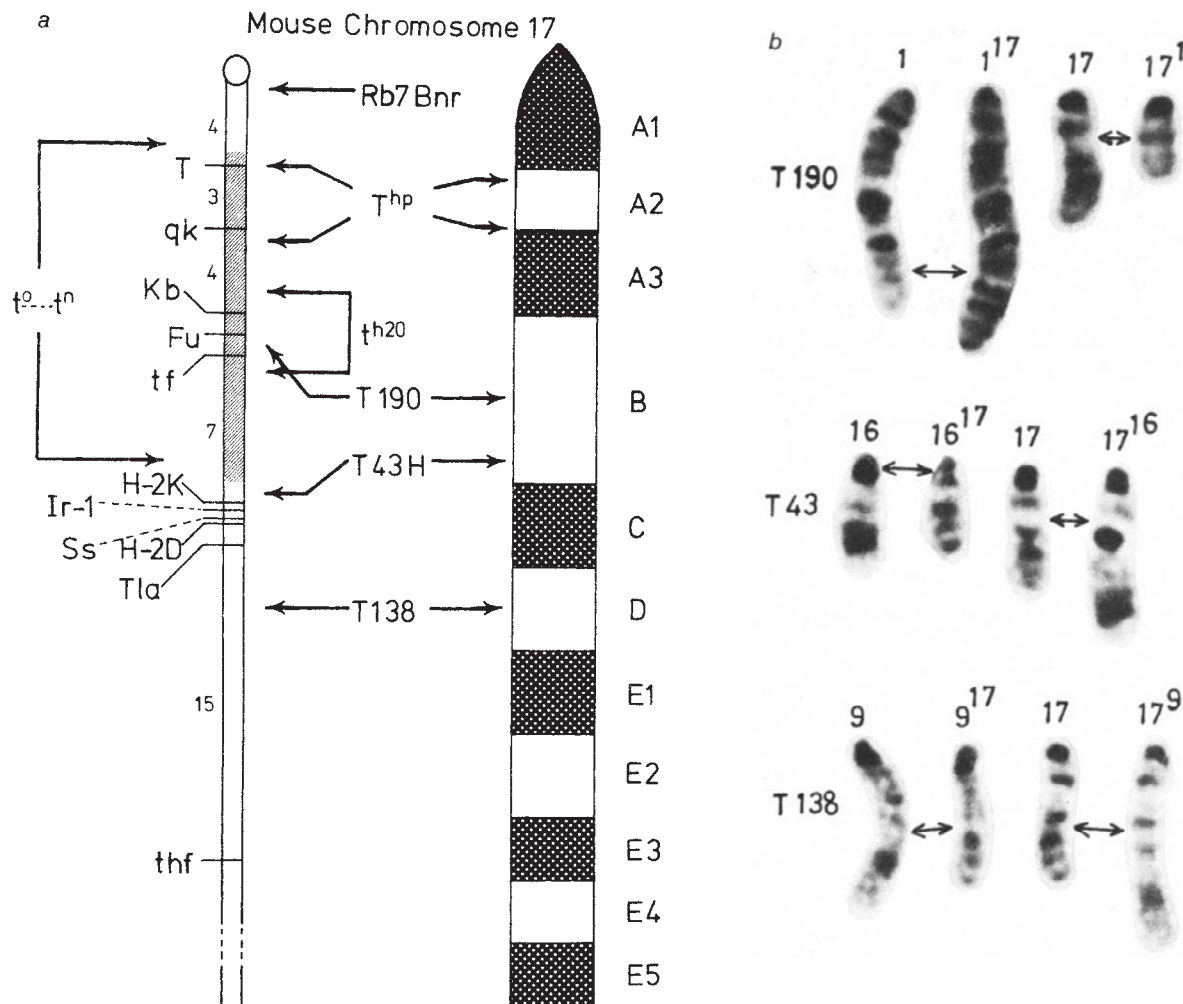


Fig. 3 *a*, Genetic (left) and cytogenetic (right) maps of mouse chromosome 17. In the genetic map the segment thought to be occupied by t-haplotypes is shaded. The approximate positions of the breakpoints in T190Ca, T43H, and T138Ca are shown by arrows. The genetic positions were determined by pedigree analysis; the evidence for the cytogenetic positions is shown in *b*. Gene symbols as in Fig. 1 and also Ir-1, immune response-1; Ss, serum serological (factor C4 of complement); Tla, thymus leukaemia antigen; thf, thin fur. *b*, G-banded normal and translocated chromosomes showing breakpoints (arrowed) in T190Ca, T43H and T138Ca heterozygotes.

Nature of the genetic changes

Although the exact mechanism remains unknown, it seems that t-chromatin alters the function of the chromosome at meiosis in such a way that chiasma formation is reduced. Moreover, as previously known, chromosome function in development is altered by t-chromatin, leading to embryonic lethality, modification of tail length and effects on spermatogenesis. In considering the type of change in the DNA which could produce such effects, it seems that major structural changes, such as inversions, are ruled out, as are long-range position effects acting from only one or two points. Such position effects could not explain the way in which effects on recombination change with length of haplotype, nor the fact that not only proximal and distal ends but also central fragments (t^{low} -mutants) can retain t-properties. It seems that some change repeated along the altered stretch of DNA in band 17B must be involved. DNA of higher organisms is thought to have five levels of organisation³⁰: (1) unique sequences, including structural genes for proteins; (2) repeated genes coding for some types of RNA and protein for which multiple coding sequences are required; (3) moderately

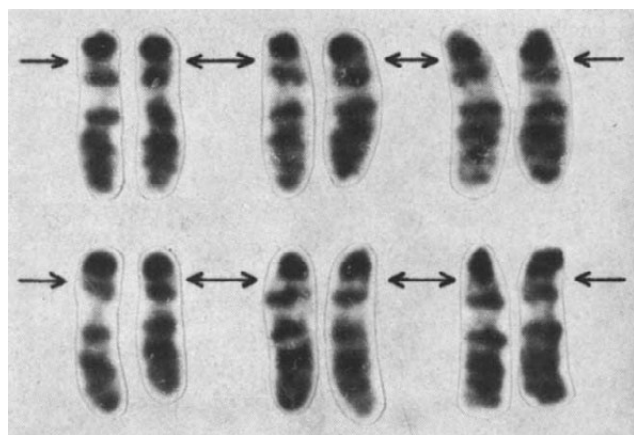


Fig. 4 Above, three G-banded homologous pairs of chromosome 17 from $+/t^{\text{h}2}$ embryos and below, three from $T^{\text{hp}}/t^{\text{h}2}$ embryos, showing no detectable heterozygosity in length or staining of the pale A2 band (arrowed).

repetitive sequences interspersed with the coding DNA (iDNA); (4) highly repetitive DNA, other than satellite DNA; (5) satellite DNA having highly repeated sequences and located in large blocks of chromatin.

We postulate that in t-chromatin it is the moderately repetitive DNA, that is, level (3) of organisation, in the chromosomal segment occupied by each haplotype, that is altered. The structural sequences in this segment seem to be present and normal. The evidence for this is that there are various known loci in the region occupied by t-haplotypes, such as the loci of qk, Kb and tf, for which t-chromatin seems to behave in a similar way to wild-type. Thus, t/qk , t/Kb , and t/tf phenotypically resemble $+/qk$, $+/Kb$, and $+/tf$, respectively. Therefore, one must assume that t-chromatin includes normal structural sequences for these loci. Conversely, a change in the iDNA could account for the observed phenomena. Such sequences are believed to be involved in the 'fine-tuning' of gene expression³⁰, and in meiotic crossing over³¹. We postulate that t-chromatin differs from normal in such a way that chiasma formation is impaired, and in development the function of different structural loci is or is not materially altered, according to their dependence on the particular change in 'tuning', which might, for instance, involve an alteration in timing or rate of transcription. Many of the effects might be quantitative, but if a processing gene³² was affected

then polypeptide structure might be altered. The lethality would result from one or more profoundly affected structural loci, the T-int effect from a slight effect on the T locus, whereas loci such as qk, Kb and tf, are not detectably affected.

Because the relevant sequences are repeated, any change in them need not necessarily be an alteration in the sequence, but might instead be an altered number of repeats, or a combination of these factors. Each naturally occurring t-haplotype, such as t^0 , $t^{\text{w}1}$, and $t^{\text{w}5}$ must be considered as potentially having its own particular type of altered iDNA. Hence, the T-int-, A- and LS-factors would potentially exist in allelic forms, according to the haplotype from which they were derived.

The means by which naturally occurring t-haplotypes arise from wild type is problematic. For repetitive sequences some mechanism, not yet understood, may exist by which a change can occur throughout a chromosome segment, such as mouse chromosome 17B, in a single event. On the other hand, it is not certain that t-haplotypes do arise in a single event. In the laboratory, short stretches of t-chromatin found in mutant haplotypes are indefinitely stable. Therefore, it is possible that in the wild, short stretches arise and become elongated by unequal crossing over until they reach a length at which they have a high transmission ratio, by which they are then maintained in the wild population. The high transmission, combined with deleterious effects (lethality and sterility), gives the impression of a 'parasitic gene' (ref. 33), and this in turn raises the possibility that incorporated viral genomes may in some way be involved in t-haplotypes. However, any explanation along these lines would be purely speculative.

Finally, if t-haplotypes are viewed as alterations in iDNA they acquire a new significance in studies of mouse genetics. Such DNA forms a significant part of the genome and if, as postulated, it affects gene expression, it would be expected to have important effects on the phenotype. Little work has so far been done on this question: t-haplotypes may provide a suitable system for future work.

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Expression in *Escherichia coli* of hepatitis B virus DNA sequences cloned in plasmid pBR322

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Fragments of hepatitis B virus DNA isolated from Dane particles have been inserted into the Escherichia coli plasmid pBR322 and cloned. Cells carrying the hybrid plasmid synthesise antigenic material that reacts specifically with antisera to hepatitis B viral antigens.

INFECTION with hepatitis B virus (HBV) is widespread in man. Between 3 and 15% of healthy blood donors in Western Europe and the US show serological evidence of past infection and about 0.1% are chronic carriers of the virus. In many African and Asian countries the prevalence is much higher and the majority of the adult population have been infected, while 5–10% of the population are chronically infected¹. Most infections are subclinical and are followed by apparently complete recovery with the development of virus-specific antibody. However, a significant proportion of infections (probably 1–5%) may produce chronic sequelae including persistent infection, chronic hepatitis of various types, cirrhosis and possibly primary liver cancer.

Plasma from some blood donors and patients infected with HBV contains 42-nm spherical particles (Dane particles)² which have serological and biochemical properties, suggesting that they are the infective virions of hepatitis B. These have an outer envelope containing the hepatitis B surface antigen (HBsAg) and an inner core (diameter 27 nm) bearing a second unrelated antigen, the hepatitis B core antigen (HBcAg). Within the core is a double-stranded circular DNA molecule of molecular weight $\sim 2 \times 10^6$ which has a large variable gap in one strand, and an endogenous DNA-dependent DNA polymerase activity that can fill in this gap in *in vitro* reactions³. There is some evidence that the total virus genome length may be around one-third greater than the 2×10^6 daltons found in single molecules in which case productive infection of a cell may require simultaneous infection by at least two genetically different particles³. A third antigen, the hepatitis B e antigen (HBeAg), which is probably also virus-coded, is found free in the plasma of some infected individuals and possibly also in association with Dane particles. Passively or actively acquired antibody to HBsAg (anti-HBs) confers some immunity to subsequent HBV challenge, and prototype vaccines composed of purified inactivated HBsAg prepared from the plasma of infected carriers are being evaluated⁴. However, the virus cannot be grown in tissue culture and normally infects only man and apes. This means that

molecular studies of the virus and its genome have been based on the limited amounts of material obtainable from the plasma of infected individuals. Such studies could be advanced considerably by insertion of HBV DNA into a bacterial plasmid or phage to allow its production in quantity from cloned, purified single molecules. Such clones would also be useful for studies of the expression of HBV gene products in bacterial cells, and for large-scale production of HBV DNA and viral antigens for diagnostic purposes and, possibly, vaccine production.

Restriction of HBV DNA

The published information on digestion products of HBV DNA (Dane particle DNA) with restriction endonucleases^{5,6} is limited and so additional analyses of this type were carried out before attempting cloning experiments. The amount of HBV DNA available was limited (about 90 ng from 5 ml of plasma), so the DNA was first labelled with ³²P in the endogenous DNA polymerase reaction³. DNA from bacteriophage λ was then added as a carrier to titrate the restriction enzyme and to provide reference fragments of known size. The digests were analysed by electrophoresis in agarose gels and the results of some of these experiments are shown in Fig. 1.

The heterogeneity of the undigested labelled HBV DNA (tracks *b* and *e*) precluded a detailed analysis of the restriction digests. The multiple bands of rather similar size could be due in part to the presence of linear and circular forms of otherwise similar molecules, in part to differing degrees of repair synthesis, although repeated DNA preparations from the same plasma sample gave reproducible patterns; the heterogeneity could also represent a true molecular dispersity from a mixed population of virions. HBV DNA preparations from several individual donors were all heterogeneous and differed slightly from each other (an example is included in Fig. 1; tracks *a*, *b* and *c* compared with *d*, *e* and *f*) both before and after digestion with various restriction enzymes. Heterogeneity has also been observed by Landers *et al.*⁶ in the products of the endogenous polymerase reaction which comprised two principal radioactive components representing linear and circular molecules with additional minor components due to incomplete repair. Examination of the HBV DNA (Fig. 1, tracks *b* and *e*) by electron microscopy showed that the population contained circular molecules of MW $\sim 2 \times 10^6$ and linear molecules ranging from 0.5 to 10×10^6 . The linear molecules represented about three times the concentration of circular molecules and only a few of them had a MW around 2×10^6 , the majority being between 0.5 and 1×10^6 . The heterogeneity observed on electrophoresis was, therefore, not due to circular and linear forms of similar length and few, if any, of the linear molecules were labelled in the polymerase reactions.

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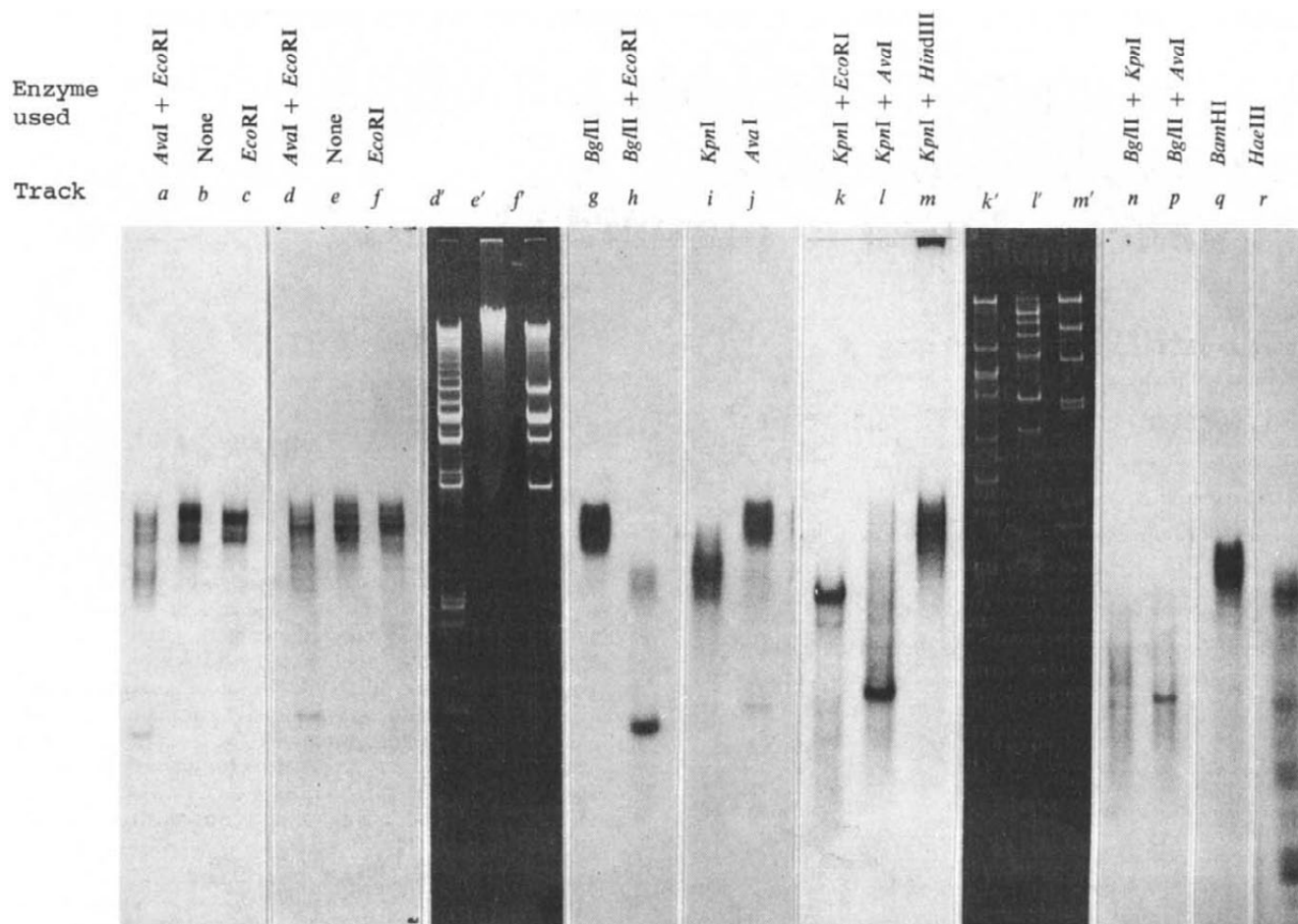


Fig. 1 Autoradiographs of restriction enzyme digests of HBV DNA after electrophoresis in agarose gels. Examples of HBV DNA isolated from the plasma of two blood donors are shown, the samples in tracks *a*, *b* and *c* from one donor being directly comparable with those in tracks *d*, *e* and *f* from the other; the sample used for the digests in tracks *d*, *e* and *f* was also used for all the other digests shown, and also for the preparative experiments for cloning. In addition to the autoradiographs, the figure includes examples, indicated by primed letters, of the gels stained with ethidium bromide to reveal fragments of the λ^+ DNA included with the HBV DNA in the restriction reactions. HBV DNA was prepared from Dane particles isolated by two cycles of ultracentrifugation from clarified plasma obtained from individual HBsAg-positive blood donors essentially as described by Landers *et al.*⁶. The single-strand gaps in the DNA molecules were repaired and the DNA radioactively labelled in reactions with the endogenous DNA polymerase in which ^3H -dCTP and ^3H -dTTP (22 and 30 mCi μmol^{-1} , respectively; Radiochemical Centre Amersham) or ^{32}P -dGTP (300 mCi μmol^{-1}) were included⁶. The released core particles were further purified by sedimentation through 30% w/v sucrose solution and DNA was isolated by treatment with proteinase K (Boehringer-Mannheim; 2 mg ml^{-1} in 0.6% SDS) followed by phenol extraction and dialysis. Restriction enzyme digests (37 °C, 1.5 h) were carried out in 10 mM Tris-HCl, pH 7.5, 10 mM MgCl_2 , 10 mM 2-mercaptoethanol, 40 mM NaCl after addition of phage λ DNA (0.5–1 μg per reaction) and the reactions were stopped by heating at 70 °C for 5 min. The samples were then applied to 1% w/v agarose gels²³ in 0.04 M Tris-acetate, pH 8.2 for electrophoresis (35 mA, 8 h). Gels were stained with ethidium bromide and photographed under UV light²⁴ and then either placed in alkali to denature DNA fragments for transfer to cellulose nitrate membrane filters¹³ or dried on Whatman 3MM paper for autoradiography. The MW of the fragments of HBV DNA was estimated from their electrophoretic mobility²⁴ with reference to fragments of λ^+ DNA in digests with *R.EcoRI* and *R.HindIII* included in the same gel²⁵. These results are included in Fig. 2 which gives a provisional map of some of the restriction targets relative to each other.

Digestion of HBV DNA with *R.EcoRI* or *R.BglII* changed the pattern of the major bands only slightly (Fig. 1 tracks *c*, *f* and *g*) whereas digestion of the DNA with these two enzymes together (track *h*) gave a radioactive fragment, MW 0.75×10^6 as the principal component, with little of the original DNA remaining. This is consistent with the introduction of a single break in circular molecules by either enzyme alone to give intact linear molecules; when the two enzymes acted together two fragments were formed, the smaller containing the entire labelled region. On digestion with *R.KpnI* (track *i*) almost the whole group of bands was displaced down the gel corresponding with the loss from each of a fragment of MW $\sim 0.4 \times 10^6$ but no corresponding radioactive fragment was found. This suggests that HBV DNA may contain at least two targets for *R.KpnI* located outside the region repaired in the reaction with DNA polymerase and spanning a sequence common to all the molecules. The alternative explanation requiring cleavage of circular molecules at a single target to give linear molecules is less likely in view of the behaviour observed in the *R.EcoRI* and *R.BglII* digests. *R.HaeIII* furnished a spectrum of fragments with a range of sizes (track *r*) in a pattern broadly similar to that published^{5,6}. Digestion of HBV DNA with *R.AvaI* and with

R.BamHI gave several radioactive fragments (tracks *j* and *g*); a major product of the *R.AvaI* digest had a MW of 0.88×10^6 , while radioactive fragments of 1.2×10^6 and 1.8×10^6 occurred in the *R.BamHI* digests; in other digests with *R.BamHI* smaller fragments were also observed. These results, together with the principal radioactive products found in various digests with pairs of restriction enzymes (for example, *R.EcoRI* and *R.AvaI*, track *a* or *d*) are summarised in Fig. 2. Within the constraints imposed by the dispersity of the HBV DNA preparations used, they provided the approximation of relative positions of restriction targets as shown.

The results imply that, in most molecules, the single-stranded gap repaired by the endogenous DNA polymerase lies within a relatively constant region of the DNA sequence; Landers *et al.*⁶ similarly concluded, from an analysis of *R.HaeIII* digests after different polymerase reaction times, that DNA repair took place largely within the same one-third to one-half of the total DNA sequence, although initiation of DNA repair could occur at variable sites within this region.

Digests of HBV DNA with *R.EcoRI* or *R.BglII* offer the possibility of cloning the entire HBV genome, while major fragments might be cloned from digests with *R.BamHI*, *R.KpnI*

or *R.AvaI*, or from various double digests. For structural studies of the HBV genome it is desirable to clone the entire DNA molecule, but clones covering a range of fragments could well be more useful for attempts to demonstrate the expression of HBV sequences in *E. coli*. Micromethods based on radioimmunoassay can be applied to individual bacterial colonies or phage plaques for the detection of specific polypeptides⁷. As many eukaryotic genes will not be expressed in prokaryotic cells it appeared desirable to insert HBV DNA fragments within a prokaryotic gene so as to produce a fused polypeptide; for this purpose the *R.Pst* target in the *E. coli* plasmid pBR322 has been used successfully⁸.

Cloning of HBV DNA fragments in pBR322

HBV DNA isolated from Dane particles from a single HBsAg positive, HBeAg positive donor (serotype *adyw*) was labelled to a low specific radioactivity with ³H in a repair reaction with the endogenous polymerase in order to facilitate its handling. This preparation was then variously digested with *R.EcoRI*, *R.BamHI*, *R.BglII*, *R.KpnI* and *R.AvaI*. Portions of the *R.EcoRI* and *R.BamHI* digests were used for insertion at the respective sites of appropriately restricted pBR322 DNA by annealing and ligation with T4 DNA ligase. The fragments from the other restriction enzyme digests and the remainder of the *R.EcoRI* and *R.Bam* digests were treated with polynucleotide terminal transferase for addition of 3' oligo (dC) sequences⁹. These fragments were annealed to pBR322 DNA to which oligo (dG) sequences had been attached after cleavage with *R.Pst*. The DNA preparations were then used to transform competent cultures of *E. coli* HB101 and transformants were screened for the acquisition of HBV sequences on the following basis. Cells transformed with recombinants made using the *R.Pst* site in pBR322 retained their resistance to tetracycline, but became sensitive to ampicillin. Cells transformed with recombinants made using the *R.BamHI* site in pBR322 became sensitive to tetracycline, but retained their resistance to ampicillin, while cells transformed with DNA cloned using the *R.EcoRI* site remained resistant to both antibiotics¹⁰ and were detected by colony hybridisation¹¹ with ³²P-labelled DNA from Dane particles. The presence of HBV DNA sequences in colonies where antibiotic resistance and sensitivities indicated the insertion of additional DNA into the plasmid was confirmed by colony hybridisation.

Characterisation of pBR322-HBV hybrids

Plasmid DNA isolated from cell cultures that had been treated with chloramphenicol to amplify plasmid production¹² was analysed by gel electrophoresis before and after digestion with restriction endonucleases.

DNA fragments from several of the gels were transferred to cellulose nitrate filters for hybridisation¹³ with HBV DNA labelled with ³²P by the endogenous polymerase reaction. Examples of these results are shown in Fig. 3 and some of the characteristics of the cloned segments are given in Table 1. The hybridisation observed with appropriate fragments does not establish conclusively that the cloned sequences were HBV-specific, for the cloned DNA and ³²P-labelled probe had been prepared from the same plasma sample. Thus contaminating non-viral DNA fragments that had been cloned inadvertently would also have been detected if the same non-viral DNA sequence was labelled by the endogenous polymerase; however, in other experiments such ³²P-labelled probes hybridised with DNA from HBV-infected liver tissue, but not with DNA from normal human liver. Furthermore, preparations of ³²P-labelled DNA made from Dane particles purified from four different blood donors gave the same patterns when hybridised against restricted DNA fragments from the recombinant plasmids, thus strengthening the view that the cloned sequences were in fact HBV DNA.

The results presented in Fig. 3 are examples taken from the large number of recombinant colonies obtained from the transformation experiments and relate to colonies described below.

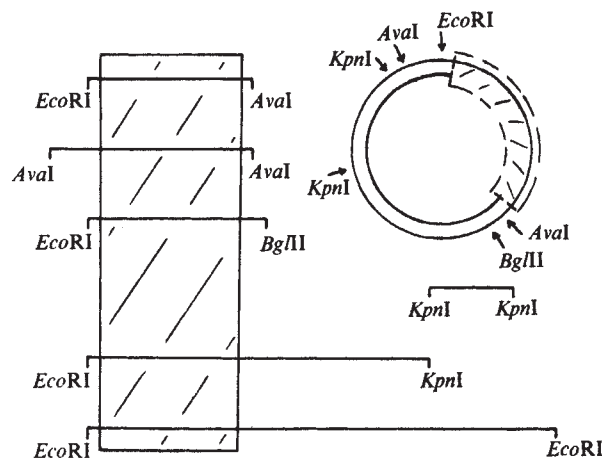


Fig. 2 The size and approximate relative order in the HBV genome of some of the major fragments in various restriction enzyme digests (Fig. 1) of HBV DNA. The site for *R.EcoRI* is taken arbitrarily as a reference point for the circular map. The shaded area indicates the region labelled with ³²P in the endogenous repair reaction.

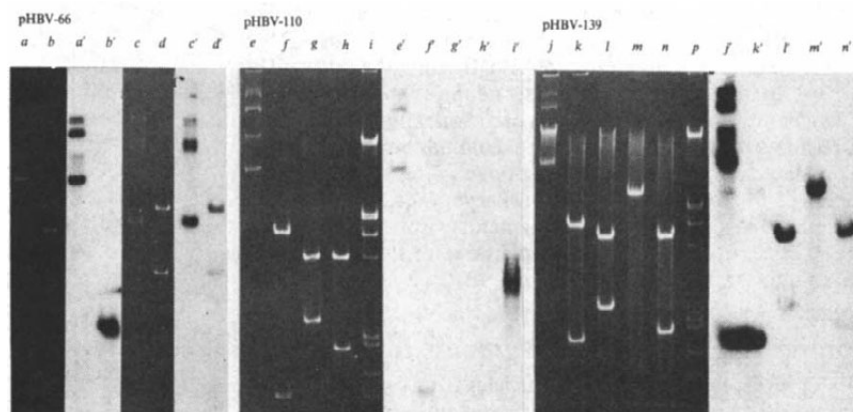
Expression of HBV sequences

A population of the colonies carrying putative recombinant plasmids, selected on the basis of drug resistance and sensitivity characteristics, was screened for the production of HBV antigens. Three test systems were used in the solid phase method of radioimmunoassay described by Broome and Gilbert⁷ which uses polyvinyl disks coated with IgG from specific antisera. These disks are placed in contact with cells producing antigen which binds to the IgG surface. When subsequently incubated with ¹²⁵I-labelled IgG from the same antiserum, the bound antigen retains the label and can be detected readily by autoradiography. The sera used were anti-HBs (human and hyperimmune animal sera), anti-HBc (human sera containing HBsAg and HBeAg and selected on the basis of a high anti-HBc titre) and anti-HBc and anti-HBe together (human sera containing HBsAg and high levels of anti-HBc and anti-HBe).

Clear positive results were obtained in the two test systems containing anti-HBc. Of some 350 colonies tested, 13 gave intense spots on autoradiography with the anti-HBc + anti-HBe antibodies (Fig. 4a). Most of these colonies remained strongly positive when re-tested in both the anti-HBc and anti-HBc + anti-HBe assay systems (Fig. 4b). None of the clones giving a negative response in the initial screening was subsequently positive, but some clones that were positive initially gave a negative result after subculturing, which probably reflects instability of some of the hybrid plasmids. All of the clones that were positive in the anti-HBc + anti-HBe system were positive when tested with anti-HBc alone, implying that none was producing detectable levels of HBeAg. If lysis of the bacterial colonies with phage λ before radioimmunoassay was omitted, only a very faint outline of the positive colonies was discernible (Fig. 4c). This is consistent with the presence of the antigen as a periplasmic polypeptide fused to the major part of β -lactamase (penicillinase) as anticipated. The sensitivity of this assay was shown to be comparable with that attainable with the same reagents in a solid phase microtitre well assay¹⁴ by titration of 10 μ l samples (diluted progressively from 1 in 400) of semi-purified HBcAg from human liver¹⁵.

The immunological specificity of the reactions was confirmed by the following observations. Replicate assays with different anti-HBc sera identified the same positively reacting clones. The positive reactions in the HBcAg assay were abolished if the polyvinyl disks were coated with normal human IgG instead of specific anti-HBc IgG, or if the normal human serum used as diluent for the ¹²⁵I-labelled antibody was replaced by anti-HBc-positive serum from a different donor (by competition with excess unlabelled anti-HBc), or if ¹²⁵I-anti-HBs replaced ¹²⁵I-anti-HBc in the assay with the anti-HBc coated disks. Finally,

Fig. 3 Electrophoretic separation in agarose gels of restriction enzyme digests of recombinant plasmids comprising pBR322 and HBV DNA sequences. The examples shown are of plasmids (Table 1) that elicit production of antigenic material that reacts specifically with antibodies to HBcAg (Fig. 4). The left-hand panels show DNA fragments revealed by staining with ethidium bromide and photography under UV light. After photography, gels were soaked in alkali for denaturation of the DNA fragments which were then transferred to cellulose nitrate membrane filters¹³ for hybridisation with ³²P-labelled HBV DNA and autoradiography; the right hand panels show the corresponding radioautographs. HBV DNA (~0.5 µg) labelled with ³H was prepared from 40 ml clarified blood plasma from a donor with a high titre of HBsAg, serotype *adwy* as described in the legend to Fig. 1 and ref. 6. In one experiment 0.5 µg *E. coli* DNA was added as a carrier, but in a second the carrier was omitted. The DNA was divided into six portions for various restriction enzyme digests. Portions of *R. EcoRI* and *R. BamHI* digests were incubated with pBR322 digested with the same enzyme in reactions with T4DNA ligase²⁶ (1 U ml⁻¹, 10 °C for 3 h followed by storage at 0 °C) in 66 mM Tris-HCl pH 7.2, 10 mM MgCl₂, 40 mM NaCl, 0.2 mM EDTA, 0.1 mM ATP, 10 mM 2-mercaptoethanol. The remainder of the *R. EcoRI* and *R. BamHI* digests as well as *R. KpnI* and *R. BglII* digests were used in terminal transferase reactions, but with the exception of the *R. KpnI* digest the samples were first incubated with phage λ exonuclease (1.5 h at 0 °C in 50 mM Na glycinate, pH 9.5, 5 mM MgCl₂, 50 µg ml⁻¹ bovine serum albumin (BSA) followed by phenol extraction and recovery of the DNA by ethanol precipitation) to remove the 5' single-stranded projections left by the restriction enzymes. Poly(dC) sequences were attached to the 3' termini by incubation with polynucleotide terminal transferase⁹ (250 U ml⁻¹ for 10–20 min at 27 °C) in 15 µl 100 mM potassium cacodylate, pH 7.0, 1 mM CoCl₂, 1 mM dCTP, 50 µg ml⁻¹ BSA, and the reactions stopped by addition of excess EDTA. An approximately molar equivalent of pBR322 DNA digested with *R. Pst* and incubated in similar reactions with dGTP instead of dCTP (from Dr J. van den Berg) was then added and the samples diluted to 50 µl for annealing in 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 5 mM EDTA, heated to 60 °C and gradually cooled to room temperature over a period of about 5 h. Aliquots of the solutions (10 µl) were then incubated with competent cultures (0.1 µl) of *E. coli* HB101 prepared as described by Lederberg and Cohen²⁷ and incubated overnight at 37 °C on L-agar plates containing tetracycline (20 µg ml⁻¹) or ampicillin (50 µg ml⁻¹). Colonies were subcultured on to Millipore filters supported on appropriate agar plates to test their resistance or sensitivity to the two antibiotics and for colony hybridisation¹¹ with ³²P-labelled HBV DNA from Dane particles. Colonies giving positive hybridisation reactions were grown in liquid culture and then shaken overnight at 37 °C after addition of chloramphenicol (170 µg ml⁻¹) to amplify the number of plasmids within the cells which were then collected by centrifugation and treated with lysozyme and EDTA¹². The resulting spheroplasts were lysed with Triton X-100 and the plasmid recovered by equilibrium centrifugation in CsCl solution (0.95 g per ml lysate) containing ethidium bromide (200 µg ml⁻¹). The plasmid bands were collected, extracted with propan-1-ol saturated with aqueous CsCl, dialysed, and samples digested with restriction endonucleases as described in the legend to Fig. 1. The results shown are from the following hybrid plasmids: pHBV-66 in tracks *a*, *b*, *c* and *d* in which *a* is the undigested plasmid and *b*, *c* and *d* are digests with *R. Pst*, *R. KpnI* and *R. BamHI*, respectively; pHBV-110 in tracks *e*, *f*, *g* and *h*, in which *e* is the undigested plasmid and *f*, *g* and *h* are digests with *R. Pst*, *R. BamHI* and *R. BamHI* + *R. EcoRI*, respectively; pHBV-139 in tracks *j*, *k*, *l*, *m* and *n*, in which *j* is the undigested plasmid and *k*, *l*, *m* and *n* are digests with *R. Pst*, *R. BamHI*, *R. EcoRI*, and *R. BamHI* + *R. EcoRI*, respectively. Tracks *i* and *p* show reference digests of λ⁺ DNA with *R. EcoRI* + *R. HindIII*²⁵ which also contained ³²P-labelled undigested DNA from Dane particles. The primed letters identify the samples on the autoradiograph, made on Kodak X-omat H X-ray film with an intensifying screen, of the cellulose nitrate filters after hybridisation with HBV DNA.



absorption of the radioactive anti-HBc with semipurified HBcAg¹⁵, by overnight incubation at 4 °C followed by centrifugation to remove the excess antigen, abolished the positive result in the radioimmunoassay. Thus the detection of an as yet unidentified HBV-specific antigen by interaction with its specific antibody present in the sera of HBV-infected individuals remains a formal possibility, but it is unlikely, and the results are wholly consistent with the observed activity being that of HBcAg. The HBcAg polypeptide detected in the colonies, however, is unlikely to be identical with that occurring naturally for the cloning experiment was such as to produce polypeptides linked to β-lactamase⁸. However, it has not been established that this is the case and it remains possible that translation of β-lactamase sequences could be terminated and followed by reinitiation, but against this is the occurrence of clones that do not exhibit HBcAg activity, but which contain HBV DNA fragments similar to those that do. The point will be clarified by DNA sequence determination, which is in progress. All of the 13 HBcAg-positive clones had been made using the *R. Pst* site in pBR322, five with fragments from *R. BamHI* digests of HBV DNA and eight from *R. KpnI* digests. Not all of the plasmids have been isolated for analysis, but the three illustrated in Fig. 3 were all from cells giving positive reactions for HBcAg. The smallest fragment of HBV DNA in these plasmids (Table 1) was about 0.95×10^6 daltons, but others with an HBV fragment

about half this size also gave positive reactions for HBcAg; values reported³ for the MW of the naturally occurring HBcAg range from 17,000 to 80,000.

In an equivalent assay for HBsAg, similar, but faint positive reactions were obtained with four clones which are being analysed further. One might expect detection of expression of serological activity to be more difficult with HBsAg than with HBcAg as its protein moiety is markedly hydrophobic and hence

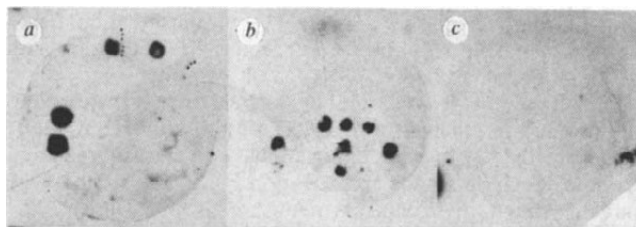


Fig. 4 Autoradiographs showing the detection by radioimmunoassay of bacterial colonies expressing HBcAg. *a*, Four HBcAg-positive colonies amongst 52 colonies examined on one plate. *b*, The result obtained when initially positively reacting colonies, together with a random selection of colonies giving a negative reaction, were subcultured from stock plates and re-tested. *c*, A duplicate of *b* in which lysis of the colonies with phage λ before radioimmunoassay was omitted. Colonies of the bacteria were grown at 37 °C overnight on Millipore filters supported on nutrient plates containing tetracycline (to maintain selection for the plasmid). The cells were lysed by imprinting the filters for a few minutes on a lawn of bacteria confluent lysed with a virulent derivative of phage λ and then incubating further for several hours at 37 °C. Polyvinyl disks coated with anti-HBc + anti-HBe specific human IgG (60 µg ml⁻¹) were placed face down on the colonies, which had obviously lysed, and incubated at 4 °C for 3–4 h. The disks were then removed and washed thoroughly and vigorously to remove the considerable quantity of adherent viscous bacterial debris. Finally the disks were incubated overnight at 4 °C with homologous ¹²⁵I-labelled IgG (10⁵ c.p.s. per µg, 2×10^4 c.p.s. per ml), washed thoroughly and autoradiographed on Kodak Blue X-ray film exposed for 2 d with an intensifying screen.

Table 1 Some properties of the plasmids shown in Fig. 3

Hybrid plasmid	MW of fragment excised by <i>R. Pst</i> × 10 ⁻⁶	Targets for restriction enzymes within the HBV sequences		
		<i>R. EcoRI</i>	<i>R. BamHI</i>	<i>R. Aval</i>
pHBV-66	1.2	—	+	+
pHBV-110	0.95	—	+	+
pHBV-139	1.16	—	+	+

In all the plasmids, the site for *R. BamHI* within the HBV sequence is located about 0.7×10^6 daltons from the *R. Pst* site near the *R. EcoRI* site.

tends to remain associated with lipid¹⁶, and some experiments (but not others) suggest that carbohydrate residues on HBsAg glycoprotein may be required for full serological activity^{17,18}.

Conclusions and further implications

A large number of hybrid DNA molecules comprising pBR322 and various fragments of the HBV genome have been cloned and propagated in *E. coli*. It thus becomes possible to produce HBV DNA in the quantities required for detailed structural and sequence analysis and location of the various coding sequences in the viral genome. Such analysis with DNA from several independent isolates and clones will explain the basis of the heterogeneity found in DNA from Dane particles. The DNA will also be useful for further genetic manipulation related to studies of expression and as a source of a highly radioactively labelled probe for hybridisation experiments for both diagnostic purposes and fundamental studies.

When inserted within a normal coding sequence of the *E. coli* plasmid the DNA from hepatitis B virus, which normally infects only man and apes, can be expressed to give serologically active translation products. This suggests that at least the region of the HBV genome coding for the amino acid sequence necessary for serological activity probably does not contain inserted sequences, or 'introns', which have now been found in a number of eukaryotic and viral genes¹⁹⁻²², for it is widely believed, although not proved, that *E. coli* cannot process transcripts of these sequences. None of the hybrid plasmids so far examined contains a target for *R.EcoRI* within the HBV sequences. It was thus possible to insert the entire plasmid into a derivative of bacteriophage λ using this restriction site and plaques of the recombinant phage gave positive reactions in the disk radioimmunoassay (results not shown). The HBV sequences may well prove more stable when propagated within the phage genome, especially as a lysogen, and appropriate exploitation of the phage regulatory systems should permit significantly increased yields of the antigens from *E. coli*, raising the possibility of large scale antigen production for diagnostic purposes and development of vaccines. The insertion of HBV DNA into a phage λ derivative has been described very recently by Fritsch *et al.*²⁸.

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letters

A 3-s delay in an optical burst from X-ray burst source MXB1735-44

THE discovery of optical bursts from the X-ray burst source MXB1735-44 was reported in a previous article¹. One burst event was detected in both X rays and optical light. We showed (in Fig. 1 of the earlier article) that the optical burst is delayed by ≈ 1.5 s relative to the X ray burst. We concluded that to within the accuracy of the SAS 3 quick-look data timing (± 1 s), the onset of the optical burst was coincident with that in X rays. A few months after the completion of our earlier paper, SAS 3 production data became available. We here report a re-analysis of the arrival times of the X ray and optical bursts based on these data which contain the universal time to an accuracy of ≤ 5 ms. We now find (as shown in Fig. 1) that the optical burst is delayed significantly by 2.8 s.

The optical observations were performed using the 1.5-m telescope at the Cerro Tololo Interamerican Observatory and a red-blocked RCA 31034 photomultiplier tube which had an

effective bandpass including the transmission of the atmosphere of 3,100-5,500 Å (FWHM). The absolute time, which was maintained to within 10 ms of UT by monitoring WWV, and the optical data in 100-ms integrations were recorded on a digital tape. Simultaneous X-ray observations were made with the horizontal tube detectors aboard the SAS 3 observatory². The three energy channels of interest are 3-6 keV, 6-12 keV and 8-19 keV which have time resolutions of 0.42 s, 0.83 s and 0.83 s, respectively. The X-ray production data are assigned absolute times in a three-step process. First, real time X-ray data and timing data from the spacecraft clock are tagged with the absolute time at the NASA ground station in Quito, Ecuador, by recording them simultaneously with the output of a UT caesium clock. Second, in the production analysis at Goddard Space Flight Center the real time data are used to assign UT to the bulk of the data which are played back from the on-board tape recorder. Third, corrections for the variable VHF propagation time from the satellite to the ground station are made using a satellite ephemeris based on four days of radar-Doppler track-

ing data. Atmospheric propagation delays limit the accuracy of the absolute timing of the production data to ± 5 ms of UT.

In the burst event shown in Fig. 1, the optical burst was delayed by 2.8 s relative to the 3–6-keV X-ray burst and ≈ 2.0 s relative to the 6–19-keV burst. Defining the moment of detection as the time of the first bin in which there is a $\geq 3\sigma$ increase above the mean background counting rate, the burst was detected first in the 3–6-keV band at 08.22.20.2 UT. The higher-energy X rays were delayed by ≈ 1 s and the optical pulse was delayed by 2.8 ± 0.5 s. The ± 0.5 -s uncertainty ($\sim 2\sigma$) in the optical delay was derived by examining alternative binning schemes for the optical and the X-ray data which have fundamental time resolutions of 0.1 s and 0.42 s, respectively. One sigma gaussian error bars are shown in Fig. 1. Also, the exact Poisson statistical significance of the first bin to exceed 3σ in each bandpass is given. The dotted line in Fig. 1 shows that the optical data are equally consistent with a 3-s linear rise to maximum from the time the burst was first detected or with a 3-s delay followed by an abrupt rise to maximum. The optical burst has about twice the duration of the X-ray burst. About half of the total optical burst flux was detected at a statistical significance of 4.0σ during the 7-s interval from 08.22.25.5 to 08.22.32.5 UT, well after the X-ray flux had ceased.

The higher energy X-ray pulses are delayed ~ 1 s relative to the 3–6 keV pulse and decay ~ 1 s sooner. The X-ray data are consistent with a black body emitter which is heated in ~ 1 s, reaches a peak temperature $\sim 30 \times 10^6$ K and subsequently cools

in ~ 1 s. This agrees with detailed studies of the X-ray spectra of several burst sources which show that the burst emission near maximum is best fitted by a $25\text{--}30 \times 10^6$ K black body^{3–5}.

The optical pulse is probably produced by the reprocessing of the X-ray pulse into light in an accretion disk or in the photosphere of an optical companion. The ~ 3 -s delay of the optical pulse may then be attributed to one or both of the following effects: (1) light travel time in the system and (2) the time required to reprocess X rays into light. (We neglect propagation delays due to the interstellar medium which are probably negligible as shown by simultaneous optical and X-ray observations of the Crab pulsar⁶.) For relatively neutral matter, in the outer portion of an accretion disk ($R \geq 1$ light s) or in the photosphere of a companion star, the reprocessing time for the bulk of the photon flux would be small (< 1 s). This is a consequence of the soft ($\sim 30 \times 10^6$ K) black body spectrum of the burst radiation discussed above. Two-thirds of the photons are below 8 keV ($\sim 40\%$ of the energy) and would be photoelectrically absorbed by C, N and O at small optical depths⁷. The third of the photons above 8 keV ($\sim 60\%$ of the energy) would be reprocessed more slowly⁷, and might contribute to the observed optical tail discussed above.

If the optical pulse originates in the atmosphere of a stellar companion, then it will be delayed due to light-travel time by an amount

$$\Delta T \approx L/c[1 - \cos \phi \sin i]$$

where L is the average distance of the X-ray star from the photosphere of the companion and ϕ and i are the orbital phase and inclination angles, respectively. We have assumed that the orbit is circular and that the stellar radius is not too large compared with the binary separation. For any i and over the range of ϕ for which a significant portion of the heated photosphere is visible ($120^\circ \leq \phi \leq 240^\circ$ for large i and the full range of ϕ for small i)⁸, $\Delta T = 3$ s gives:

$$1.5 \text{ light s} \leq L \leq 6 \text{ light s}$$

or

$$0.6 R_\odot \leq L \leq 2.6 R_\odot$$

The model predicts an observable modulation ($\geq 25\%$ for $i \geq 15^\circ$) of the delay time at the orbital period. As we noted in our earlier paper¹, the lack of photometric variability (< 0.1 mag) in the steady optical source^{9,10} argues against this model and suggests that an accretion disk may contribute significantly to the optical luminosity and/or shield much or all of the stellar surface.

Alternatively, the optical pulse could be produced in the outer portion of an accretion disk ($R \sim 2\text{--}3$ light s). In this case the time delay would show at most a small modulation at the orbital period (see DQ Her in refs 11, 12). It may also be variable, if for example, the burst emission is beamed or if there are changes in the structure of the disk.

The optical pulse might also originate in the fully ionised inner portion of an accretion disk ($R \leq 1$ light s) which is not shielded from the compact source and in which the reprocessing time is long. The results of steady-state calculations for uniform density models scaled to high densities^{13,14} and for models of X-ray illuminated atmospheres extrapolated to high fluxes¹⁵ suggest that irradiated matter within ~ 1 light s of the compact source will be thick to Thomson scattering and thin to photoelectric absorption. In these conditions the burst X rays which are not reflected from the disk will undergo many Thomson scatterings, and the bulk of the optical photons will be produced at large optical depths. Detailed calculations are required to estimate how these and other reprocessing effects would determine the delays, widths, rise times and intensities of optical bursts.

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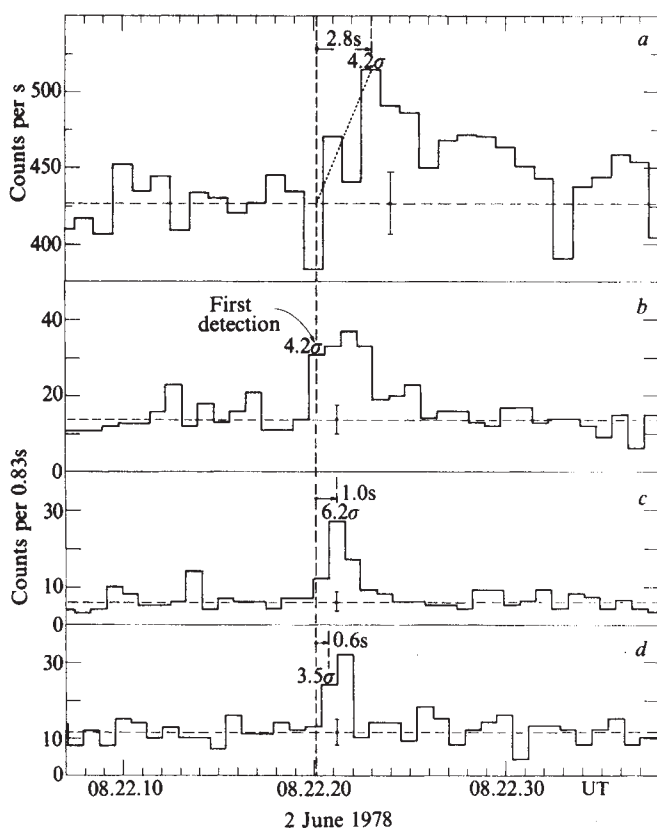


Fig. 1 X-ray/optical burst from the X-ray burst source MXB1735–44. The event was detected first in 3–6-keV X rays, as marked by the vertical dashed line, and ≈ 2.8 s later in blue light. The background counting rates and their approximate ($N^{1/2}$) uncertainties are indicated by horizontal dashed lines and error bars, respectively. The exact Poisson statistical significance of the first bin to exceed a 3σ detection threshold is also given. The dotted line shows that the optical data are equally consistent with a 3-s linear rise to maximum from the time the burst was first detected or with a 3-s delay followed by an abrupt rise to maximum. A large tail is apparent in the optical data (see text). *a*, Optical 3,100–5,500 Å; *b*, 3–6 keV; *c*, 6–12 keV; *d*, 8–19 keV.

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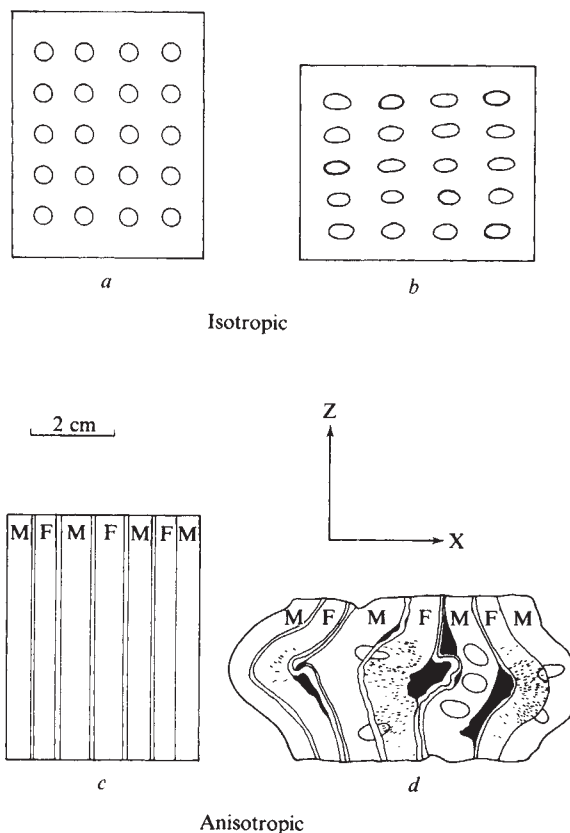


Fig. 1 *a*, An isotropic block of fine-grained ice with circular strain markers. *b*, The isotropic sample after 21% shortening and the strain markers are now ellipses. *c*, The planar fine- and medium-grained layers in the anisotropic block, separated by the films of frozen water. *d*, The anisotropic ice after 36% shortening. Voids have developed between layers (black), and air bubbles are more elongate (fine lines) in the hinge region of the fine-grained layers. Only a few elliptical strain markers have been preserved on this XZ face after deformation.

Experimental folding in ice and the resultant *c*-axis fabrics

FOLDS are commonly observed features of naturally deformed rocks¹ and glaciers^{2,3}. Many different materials⁴ have been used to model naturally occurring fold patterns. The deformation experiment described here used ice as an analogue for quartz in quartz-rich rocks by deforming a sample with an initially planar layering or anisotropy. This deformation is compared with samples of unlayered isotropic polycrystalline ice (Fig. 1*a*). Ice is convenient to use in the laboratory because of its relative ease of deformation at readily controlled temperatures. When used to model the behaviour of quartz-rich rocks, ice is a realistic analogue because both ice and quartz have: (1) hexagonal crystal structures; (2) exhibit similar optical properties; (3) form polycrystalline aggregates; and (4) deform using common crystallographic slip planes, particularly the (0001) basal planes^{5,6}. In our experiments, a sample of multilayered ice consisting of plates of fine-grained ice sandwiched between layers of coarser ice (Fig. 1*c*) was deformed in a plane strain apparatus. The *c*-axis orientations of the grains were initially random and therefore the composite sample resembled the structure of many naturally occurring quartzites⁷. The experimental technique described here makes it possible to explore the relationship between initial anisotropy on folding and fabric development.

Cylinders of polycrystalline ice were prepared from a mixture of sieved ice-crushings and distilled water (details of the method will be published elsewhere). The ice contains equant grains of essentially uniform grain size with randomly orientated *c*-axes. The grain size can be controlled and for these experiments cylinders with grain sizes of 1 mm (fine grained) and 2 mm (medium grained) were produced. An irregular distribution of small air bubbles between 0.1 and 0.2 mm diameter were

present at an estimated concentration of 10 mm^{-3} , in the grain boundary areas, in parts of the fine-grained ice.

Each cylinder was cut into plates ~6 mm thick and also a block ~50×50×60 mm. A composite layered block of comparable dimensions was made from the two sets of plates. After being cut flat on a Leitz microtome, the surfaces of each plate were coated with a 1% solution of polyvinyl formate (Formvar) dissolved in ethylene dichloride and allowed to dry for 12 h. The surfaces were then frozen together with a film of distilled water to produce an anisotropic block of alternate fine- and medium-grained layers (Fig. 1*c*).

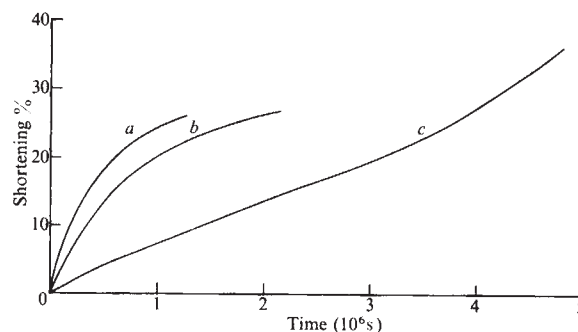


Fig. 2 Displacement-time curves for blocks of fine-grained (*a*) and medium-grained (*b*) isotropic ice shortened by 27% and anisotropic ice (*c*) shortened by 36%.

Strain markers consisting of circular grooves 5 mm diameter, 0.4 mm deep and 0.3 mm wide, were inscribed at 10 mm centres on the XZ faces of the three blocks. (Here the extension direction is X , the direction of no length change Y , and Z is the shortening direction). The blocks were then deformed in a plane strain apparatus similar to those described by Kamb⁸, and Budd and Matsuda⁹. The layers in the composite block were parallel to the YZ plane. The load on the layered block was 83 kg and that on the isotropic samples 150 kg and so the initial stresses were 0.4 MPa and 0.6 MPa respectively. During loading and unloading the temperature was typically -10°C , and for the deformation it was controlled to $-1.00 \pm 0.05^\circ\text{C}$. The displacement-time curves of the fine-grained and medium-grained isotropic ice are of a similar decelerating form, whereas the more lightly loaded anisotropic sample deformed at an increasing rate after 20% shortening (Fig. 2). This is presumably attributable to buckling and increasing instability of the layers.

The isotropic ice deformed homogeneously on a scale greater than a few grain diameters. The numerous small amplitude undulations (parallel to Y) that formed on the YZ faces of both the fine- and medium-grained samples are the only inhomogeneities observed, and have amplitudes and wavelengths of the order of the average grain size. The uniform distortion of the circular strain markers into a set of ellipses suggests that extension in the X direction was uniform. The bubbles in the fine-grained sample were elongated parallel to the strain ellipses. Grain sizes were uniform and boundaries were slightly irregular. Occasional deformation bands developed in individual grains as sharply delineated, wide lamellae. The c -axis orientation patterns of deformed fine- and medium-grained isotropic samples were similar (Fig. 3a and b), and were found not to vary throughout each sample. The majority of c -axes are distributed between the 25 and 45° cones centred about the shortening axis. This pattern is also consistent with other plane strain experiments with shortenings of greater than 40% (unpublished data).

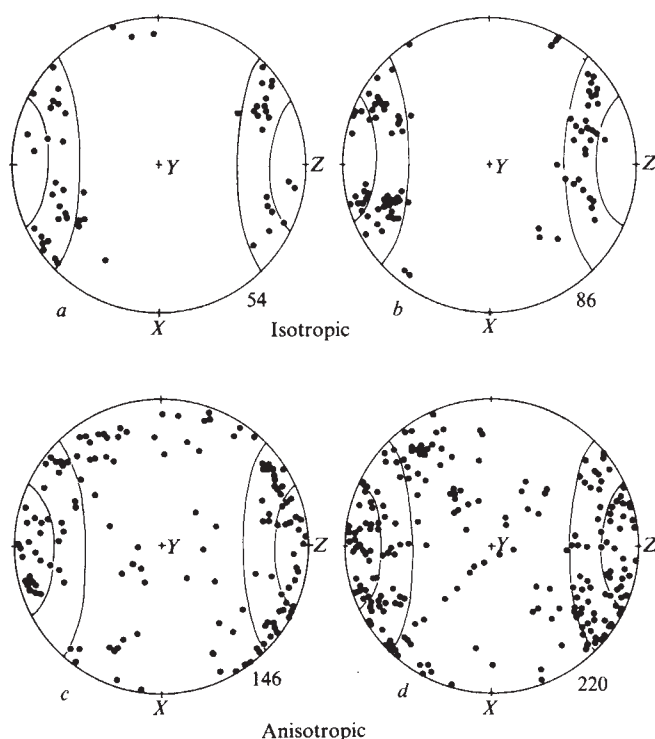


Fig. 3 c -axis patterns of preferred orientation. (a) and (b) show the fine- and medium-isotropic ice shortened by 27%. (c) and (d) show the fine and medium layers in the anisotropic ice shortened by 36%. The data are plotted on the lower hemisphere equal area projection and the superimposed small circles are at angles of 25° and 45° to the shortening axis Z . The number of c -axes is shown adjacent to the projection.

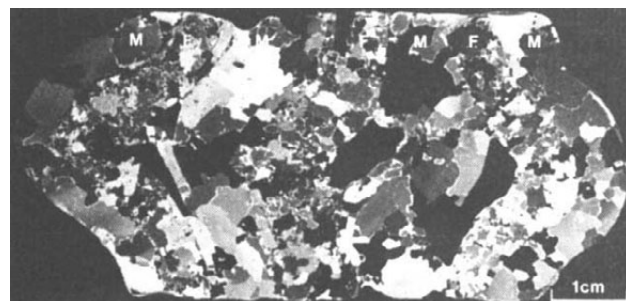


Fig. 4 Microstructure in the folded fine- and medium-grained layers of the anisotropic ice. An interlocking grain aggregate in the fine layers is in marked contrast to the large grains of the medium layers.

The anisotropic specimen deformed inhomogeneously and large symmetric sinusoidal folds were produced in the medium- and fine-grained layers (Fig. 1d). The folds are disharmonic and their axial surface lies approximately perpendicular to the direction of shortening. Folds in the outer layers are parallel and penetrative, and their hinge lines make a 20° angle to Y (in the XY plane). Fold amplitude and sense of closure change in the Y direction in the central portion of the block, where dilation voids also appear between layers. The thin ice films used to bond the fine- and medium-grained layers together have also been folded, partly in conformity with adjacent fine- and medium-grained layers. However, adjacent to the voids the ice films display minor folding where axial surface orientations vary from perpendicular to parallel to the shortening direction.

The strain distribution within individual layers is inhomogeneous with the strain ellipses being distributed about the axial surface of the major folds. Where an original circle lay across the boundary between a fine- and medium-grained layer it was either significantly elongated in the fine layer or displaced parallel to the layer boundary. The small air bubbles in the fine-grained layers are elongate and describe a symmetric fan-like pattern.

In the anisotropic ice sample the relative grain size difference between adjacent layers has been preserved. Grain shapes are quite irregular and grain diameters have increased (Fig. 4). In the fine layers the average grain size is 1.5 mm, and in the medium layers grains vary from 2.5 to 4.5 mm. Nearly every grain contains undulose extinction or deformation bands. The c -axes in both the fine and medium layers (Fig. 3c and d) are distributed throughout the projection with a strong maxima parallel to the shortening direction. Such a pattern is a significant departure from the small circle distribution of the isotropic samples. An axial distribution analysis¹⁰ of the anisotropic samples suggests that there is no relationship of c -axis orientation to relative position in the fold. The pattern of c -axes is consistent with some patterns recognised in natural⁷ and experimentally deformed¹¹ polycrystalline aggregates of quartz and also natural ice¹².

Folding induced by an initial layering produces a different fabric from a homogeneously deformed polycrystalline aggregate. This effect on fabric development has not been considered in other experimental studies of either ice or quartz, nor in any of the computer simulations of fabrics^{13,14}. The displacement of strain markers between layer boundaries, the variable axial-surface orientations associated with the minor folds, and their partial parallelism to the layer boundaries, suggest that during the deformation there has been some degree of layer boundary slip. Therefore buckling and flattening proceed simultaneously with layer boundary slip and a component of plane strain. This compares markedly with the isotropic samples where the deformation is strictly a homogeneous plane-strain deformation. It is the layering then that is crucial in determining the deformation path of any point in the material.

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Thiocyanate in Red Sea brine and its implications

THE Oparin–Haldane hypothesis about the origin of life has provided a rational theoretical model for laboratory research. Almost thirty years of experiments have supported this hypothesis. Verification of the hypothesis in terrestrial environments, however, has not been documented. Some environments such as thermal springs in the Kuril Islands have been reported to have possible chemical precursors but lack of sterility and other factors have not made it possible to establish evidence for natural, terrestrial abiotic synthesis¹. We identify here the Atlantis II Deep brine as a promising site for searching for chemical precursors, report finding thiocyanate in the brine and suggest a relationship between chemical evolution and the evolution of the Earth's crust.

We have recently suggested² that the Red Sea brines, located at the bottom of the sea in the rift valley of an active axis of spreading global plates, might be a fruitful place to search for terrestrial evidence of abiotic synthesis of life precursors². The Atlantis II Deep brine (21°22' N, 38°05' E) was thought to be especially promising because: (1) it has been reported sterile³; (2) it has a methane enrichment 10^3 times normal seawater⁴; (3) it has no free oxygen (O_2)⁵; (4) it is unusually warm (63 °C) (D. A. Ross, personal communication); and (5) it has been reported as a 'reducing' environment⁵.

During Cruise 93, Leg 19 of the RV Atlantis II in spring 1977 the Atlantis II Deep brine was identified by seismic profiling and depth recording revealing a brine of about 200 m thickness at a depth of 2,000 m. Water samples were collected using 10 l and 30 l Niskin samplers set at 7 m intervals from 14 m above the bottom to 225 m above the bottom at Station 87. Our samples were subsequently stored in sealed, sterilised, brown, opaque Nalgene bottles and thymol crystals were added to half to ensure continuous sterility. Sterility of the samples taken at this station from the brine has been confirmed (R. Cuhl, personal communication).

Aliquots (5 cm³) of Bottle no. 1 (30 l) from Station 87 were chromatographed using butanol/ammonia/water on Whatman no. 1 paper⁷. The R_f of known thiocyanate was 0.25 in these conditions. Strips of 4 cm length corresponding to the R_f of known thiocyanate were cut, eluted with 5 cm³ of distilled water and flash evaporated to their original volume. Aliquots of 0.5 cm³ were tested for thiocyanate using 0.3 cm³ 0.1 M cupric chloride solution and 0.5 cm³ of a ferric nitrate/nitric acid reagent. Thiocyanate produces a wine red colour with these reagents and a spectrophotometric absorbance peak at 4,600 Å

(ref. 8). (This analytical method was developed to measure thiosulphate by adding cyanide to produce sulphite and thiocyanate. The reagents are added to produce a ferric thiocyanate complex. The thiosulphate concentration corresponds to the absorption of the complex. We used this method as a direct measure of thiocyanate. Thiosulphate without cyanide produces no spectrophotometric absorbance with these reagents.)

The Atlantis II Deep brine samples were analysed against a 'null' brine of 5M NaCl, 0.2M KBr and 0.02M KHCO₃ on a Zeiss ZFM 4/v4QIII/PMQII spectrophotometric system and a Unicam SP80 recording spectrophotometer. The spectra of the samples were scanned from 4,300 Å to 6,000 Å and compared with the spectrum of known ferric thiocyanate complexes with respect to general shape and the absorption ratios at several wavelengths. Aliquots (5 cm³) were chromatographed, eluted and concentrated to one-tenth the original volume and analysed. Results of the direct analysis confirm a thiocyanate concentration of 2.4×10^{-5} M (mean of 12 spectrophotometric analyses; standard deviation = 1.2) in the Atlantis II Deep brine. No thiocyanate was detected in a control sample of Red Sea water.

In four experiments, a known amount of thiocyanate was added to the brine to determine whether this would increase the spectrophotometric absorption corresponding to the amount added. This was confirmed in all four experiments. Results of these experiments yield a mean thiocyanate concentration of 2.8×10^{-5} M (range 2.3 – 4.0×10^{-5} M) in the brine. This concentration is significant from the point of view of prebiotic chemistry.

Some samples were also treated with Na₂S₂O₃ before analysis to test for the presence of cyanide ions⁸ but none were found. All cyanide, however, would be expected to convert to thiocyanate since the underlying sediments through which the brine flows are rich in elemental sulphur and sulphides such as pyrite, chalcocopyrite, sphalerite and marcasite^{9,10} making the environment similar to conditions for the commercial preparation of thiocyanate from cyanide.

What is the origin of the thiocyanate in the Atlantis II Deep brine? No marine organisms are known to release cyanide or thiocyanate into the marine environment. Both cyanide and thiocyanate have been reported from geothermal springs¹. Hydrogen cyanide may have been formed abiotically and released from within the ridge system after which it reacted with sulphur or sulphides to form thiocyanate. We think this is the most likely source of the thiocyanate.

The important role of hydrogen cyanide and its related chemical species in the prebiotic synthesis of purines, pyrimidines and amino acids is well-documented from Miller¹¹ to Ferris and Joshi¹². The discovery of thiocyanate in a sterile, natural terrestrial environment such as the Atlantis II Deep brine is consistent with our suggestion that there may be a relationship between the evolution of the Earth's crust at spreading axes and the origin of life. While the presence of thiocyanate in a sterile brine does not equivocally demonstrate the abiotic origin of the cyanide from which the thiocyanate may derive, it suggests that further analysis of the Atlantis II Deep brine should be undertaken to assess the character of organic molecules present. Previous analysis of hydrocarbons in the brine has indicated pronounced enrichment in methane, ethane and low molecular weight paraffins³. Abiotic synthesis of amino acids in this environment is plausible.

Criteria for determining whether amino acids were formed biotically or abiotically include the D and L enantiomer ratio, the ¹³C and ¹²C ratio and the type of amino acids present¹³. As the brine has a high temperature, the D and L enantiomer ratio may not be a useful criterion. Amino acids such as α-aminoisobutyric acid, α-amino-n-butyric acid, N-ethylglycine, N-methylalanine, norvaline and sarcosine in amounts similar to those found on the Murchison meteorite would suggest an abiotic origin. We intend to search for amino acids in the Atlantis II Deep brine and will use these criteria in our evaluation.

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Origin of carbonatites by liquid immiscibility

THE existence of carbonatite magmas has been generally accepted¹, but their origin remains uncertain. The more favoured petrogenetic models include: (1) direct partial melting of the upper mantle^{2–5}; (2) fractional crystallisation of CO₂-rich alkaline silicate magma⁶; and (3) separation of an immiscible carbonate melt from an initially homogeneous CO₂-rich alkaline silicate magma^{7–10}. Experiments have shown all of these processes to be feasible^{5–7}, and each may generate the geochemical characteristics of carbonatite, such as enrichment in rare earths and other incompatible trace and minor elements^{11,12}, and low ⁸⁷Sr/⁸⁶Sr ratios¹³. Here we discuss the role of immiscibility, and report new experimental data which demonstrate for the first time that liquid immiscibility does occur between silicate and carbonate liquids of the compositions found in nature.

While previous experimental work suggests that high concentrations of Na₂O are required for immiscibility^{7,14,15}, the great majority of analysed carbonatites are rich in CaO with Na₂O as only a minor constituent, seemingly ruling out a role for immiscibility in their genesis (for example, mean of 12 Kenyan alvikites CaO = 50.83, Na₂O = 0.48 wt % ref. 9, p. 317). However, it has been argued that the present-day compositions of most carbonatites are not representative of the original magmas but have lost large quantities of alkali oxides during fenitisation and due to leaching by meteoric waters^{2,3,9,16,17}. This view is supported by the study of inclusions in minerals from carbonatites, which show that alkaline CO₂-rich fluids were present at high temperatures^{18–20}. It is significant that the only carbonatite which has been observed at the time of eruption, the natrocarbonatite of Oldoinyo Lengai, Tanzania^{21–25}, contains about 30 wt % Na₂O (Table 1). This lava has retained its alkalis

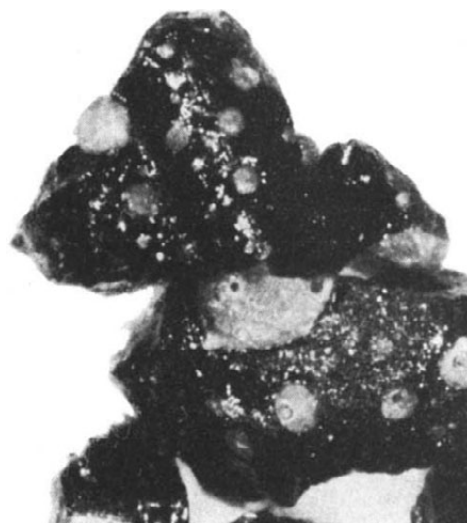


Fig. 1 Spheres of carbonate in silicate glass. The diameters range up to about 2 mm. The small pits seen in the carbonate are probably CO₂ vesicles. Starting material was 15% carbonate plus 85% nephelinite, run conditions 2 kbar, 1,000 °C for 18 h.

because it reached the surface and quenched, precluding fenitisation processes, and because it was sampled before it had been leached. Xenoliths of plutonic carbonatite from the same volcano are CaCO₃-rich sövite²⁴. The main aim of the present study was to investigate the possibility of an immiscibility relationship between the Oldoinyo Lengai natrocarbonatite and the contemporaneous silicate magmas.

Two synthetic carbonatite compositions were made up, one a simplified version of the Oldoinyo Lengai lava, and the other richer in CaCO₃ (Table 1). These were mixed and ground in various proportions with two powdered silicate lavas from Oldoinyo Lengai, a phonolite and a nephelinite (Table 1). About 0.7 g samples of dried powder were sealed in noble-metal tubes and run at pressures from 0.7 to 7.6 kbar and temperatures from 900 to 1,250 °C in argon-filled internally heated pressure vessels. Selected run data are given in Table 1.

In general, charges which were not of extreme composition (that is, very close to either silicate or carbonate starting materials) showed characteristics on quenching consistent with the coexistence of two melts at the pressure and temperature of the experiment. Silicate melts quenched to glasses, which were separated by smooth, sharp menisci from carbonate melts, which quenched to crystalline aggregates. The actual geometry of the association depended on the proportion of carbonate in the starting mixture, and the temperature and pressure of the run. Where the proportion of silicate greatly exceeded that of carbonate, globules of carbonate were observed in silicate glass, see, for example, Fig. 1. Where there was a moderate to high proportion of carbonate, however, (≥40 wt %), a central ovoid slug of silicate glass normally occurred, flanked by ends of carbonate. Depending on temperature, a small number of globules of one phase was observed in the other, but at temperatures ≥1,000 °C almost complete separation of melts took place.

Where a significant amount of crystals are present in the silicate melt the physical separation of the two melts is not so perfect. In 50/50 original mixtures of silicate and carbonate the final geometry of the charge was still a central lozenge of silicate glass with two end cappings of carbonate but significant amounts of carbonate globules occurred throughout the silicate glass. The larger volumes of carbonate remain essentially silicate-free, however, and it was easy to hand pick a silicate-free carbonate fraction. This was analysed by wet chemical methods. The silicate glass was analysed by electron microprobe. Microprobe

analysis of the carbonate fraction using a defocused beam generally gave broadly comparable results with the wet chemical analysis, but was considered less reliable because of the instability of the carbonates. However, it did allow some supplementary data to be obtained on elements which were not analysed wet chemically.

Several features suggest that the phases we have analysed represent equilibrium immiscible melts and are not just the result of the failure of the starting mixtures to homogenise. These include: (1) scanning electron microscope study of the charges shows that the meniscus between the melts is sharp on a submicron scale; (2) the silicate glasses are compositionally homogeneous throughout the slugs; (3) spheroids of silicate glass, ~100 μm diameter, in the quenched carbonate melts have compositions identical to those of the main silicate slugs; (4) spheroids of quenched carbonate liquid in the silicate glass, a few tens of μm diameter, yield microprobe analyses which are comparable to the wet chemical analyses of the main carbonate fraction, bearing in mind their instability in the electron beam. We have also carried out reversal type experiments to test for gross metastability. In the first instance, two melts were held at conditions where the miscibility gap is narrow (high T , low P), so that the silicate dissolved large amounts of carbonate. The homogeneous silicate-glass fraction was removed and re-run in conditions where the miscibility gap is wide (low T , high P) and it exsolved small spheres of carbonate. Second, it was found that silicate glass containing a few spheroids of carbonate (~0.1 mm diameter), produced at high P and low T could be homogenised by re-running at low P and high T . These experiments demonstrate that the carbonate melt would readily dissolve, or exsolve from the silicate melt in the conditions of interest in this study.

The main characteristics of the immiscibility field encountered in this study are shown in Table 1 and Fig. 2. The components of Fig. 2, $\text{SiO}_2 + \text{Al}_2\text{O}_3$, $\text{Na}_2\text{O} + \text{K}_2\text{O}$, CaO , represent ~90% of the CO_2 -free portions of both melts. Most of the data are for one temperature, 1,100 $^\circ\text{C}$, but for three pressures, 0.7, 3.0 and 7.6 kbar. Conjugate melts at both 0.7 and

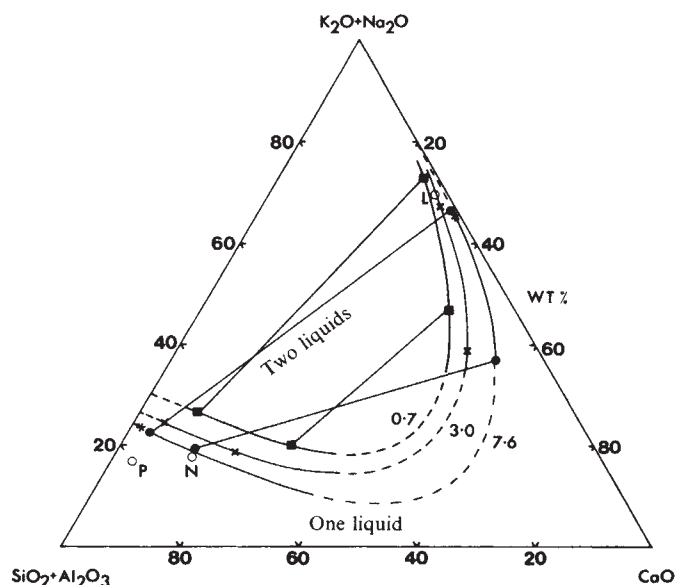


Fig. 2 Miscibility gap between carbonate and silicate melts. Conjugate melts are shown for 0.7 kbar, 1,100 $^\circ\text{C}$ (■); 3.0 kbar, 1,100 $^\circ\text{C}$ (×); 7.6 kbar, 1,100 $^\circ\text{C}$ (●); and 3.0 kbar, 900 $^\circ\text{C}$ (*). The boundary between the one-liquid and two-liquid fields has been drawn for each pressure and 1,100 $^\circ\text{C}$. Conjugate liquids at 0.7 kbar and 7.6 kbar are joined by tie-lines. The liquids furthest from the CaO apex were produced from mixtures of phonolite and CaO -poor carbonatite, those closer to the CaO apex were produced from mixtures of nephelinite and CaO -rich carbonatite. P, Oldoinyo Lengai phonolite; N, nephelinite; L, natrocarbonatite.

Table 1 Starting materials and run products

	1	2	3	4	5	6	7	8
SiO_2	41.93	2.00	44.46	4.5	52.88	—	52.23	0.16
TiO_2	0.92	—	0.73	NA	0.93	—	0.71	0.18
Al_2O_3	15.55	—	14.68	0.40	19.89	—	17.41	0.5
Fe_2O_3	6.41	—	—	—	4.07	—	—	—
FeO	2.28	—	5.41*	2.30*	1.58	—	2.99*	1.54*
MgO	1.28	1.00	1.05	0.92	0.45	—	b.d.	0.76
CaO	10.89	33.32	11.13	31.59	2.67	16.69	1.96	21.82
Na_2O	10.26	16.66	11.07	16.35	10.63	33.38	13.28	27.00
K_2O	4.95	5.55	5.73	5.16	4.91	8.34	6.95	5.53
P_2O_5	0.54	1.00	0.23	1.21	0.11	1.00	b.d.	1.01
CO_2	2.39	35.47	NA	NA	0.15	35.59	NA	NA
F	NA	2.00	NA	NA	NA	2.00	NA	NA
Cl	NA	3.00	0.48	NA	NA	3.00	0.19	NA
Others	2.28	—	—	—	1.77	—	—	—
Totals	99.68	100.00	94.97	62.44	100.04	100.00	96.72	58.50

1, Nephelinite BD119, Oldoinyo Lengai. Analysis includes $\text{H}_2\text{O}^+ = 0.80$, $\text{H}_2\text{O}^- = 0.85$.

2, Synthetic high- CaCO_3 carbonatite.

3, Immiscible silicate melt produced by melting equal weight mixtures of 1 and 2 at 7.6 kbar, 1,100 $^\circ\text{C}$. (● in Fig. 2.)

4, Carbonate melt in equilibrium with 3. (● in Fig. 2.)

5, Phonolite BD50, Oldoinyo Lengai.

6, Synthetic carbonatite, a simplified mean of Oldoinyo Lengai lavas, with $\text{Na}_2\text{O}:\text{CaO}:\text{K}_2\text{O} = 4:2:1$.

7, Immiscible silicate melt produced by melting equal weight mixtures of 5 and 6 at 3 kbar, 900 $^\circ\text{C}$. (* in Fig. 2.)

8, Carbonate melt in equilibrium with 7. (* in Fig. 2.)

NA, not analysed; b.d., below detection limits of energy dispersive microprobe (~0.3 wt %).

Analysts: T. Jensen, carbonate run products, I. Freestone, silicate run products, D. G. Powell, lavas.

* Total iron as FeO.

7.6 kbar are joined by tie-lines. The data points for each pressure are joined by curves which represent the isothermal, isobaric phase boundaries between the one-liquid and two-liquid fields (Fig. 2). The data indicate that the miscibility gap closes towards the CaO apex; this is supported by data on other systems, for example, immiscibility was not found in $\text{NaAlSi}_3\text{O}_8\text{--CaCO}_3\text{--H}_2\text{O}$ at 1 kbar (ref. 6). Therefore, we have extrapolated the field boundaries and shown the closure as dashed lines (Fig. 2). Comparison of the boundary curves shows that increasing pressure expands the miscibility gap. The inclusion on Fig. 2 of a run at 3 kbar and 900 $^\circ\text{C}$ shows that decreasing temperature expands the two-liquid field in a manner analogous to increasing pressure. It is stressed, however, that the effect of changing P or T is not just a simple narrowing or widening of the gap but complicated changes in composition take place including rotation of the tie-lines.

The compositions of Oldoinyo Lengai phonolite (P), nephelinite (N) and carbonatite (L) are also shown in Fig. 2. The position of the tie-lines suggest that the natrocarbonatite is likely to have been immiscible with a phonolitic magma at high pressures and temperatures. Nephelinite melt, on the other hand, was consistently found to be immiscible with carbonate melts much richer in CaO than the Oldoinyo Lengai lava (Table 1, Fig. 2). Therefore the experimental study supports the view²⁵ that the Oldoinyo Lengai carbonatite exsolved from a CO_2 -rich phonolitic magma.

Most natural carbonatite compositions lie close to the CaO apex of Fig. 2, and it seems unlikely that the tie-lines could rotate sufficiently for phonolitic or nephelinitic magmas to be conjugate with carbonate melts so poor in Na_2O , even at very high pressures. If these carbonatites did originate by immiscibility, then significant loss of alkalis must have occurred at some stage in their development, as proposed above. The failure of many experimentalists to observe immiscibility during experiments on CO_2 -rich systems at pressures to 30 kbar^{6,14,15}, or to confirm the presence of immiscibility which had been tentatively identified^{26,27}, is because the compositions studied were poor in

Na₂O, and plotted in the one-liquid field of Fig. 2. However, the compositions of many mafic and ultramafic alkaline igneous rocks, such as melanephelinites, melilitites, kimberlites and alnoites, also project into this field, suggesting that the corresponding magmas are unlikely to exsolve carbonate melts at crustal pressures, although immiscibility in the mantle remains a possibility.

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Variation in apatite composition in ijolitic and carbonatitic igneous rocks

We present here evidence that apatite crystals formed in alkaline magmas during the early stages of both the ijolitic (pyroxenite → ijolite → urtite) and carbonatitic (sövite → alvikite) differentiation series¹ have similar chemical compositions, but that with subsequent crystal differentiation in both series, the compositions of the apatites can change progressively along two separate paths. The evidence of a bifurcating pattern of apatite compositions from the various rock types studied is not in accord with the theory^{2,3} that carbonatites are the ultimate product of fractional crystallisation of the ijolitic series. The evidence suggests that ijolite and sövite are the crystalline products of conjugate immiscible liquids.

Any wet chemical method of apatite analysis relying on mineral separation techniques introduces the possibility of contamination⁴, and hence many published apatite analyses must be considered suspect. Electron microprobe (EMP) analysis, however, can provide good quality data, and minor chemical variations can be used as reliable indicators of fractionation processes⁵. Our data show greater variation in chemical composition than was previously indicated⁶.

The means of 115 EMP analyses of apatites in Table 1 are taken from five associated rock types from two adjacent well documented strongly alkaline igneous complexes¹. Very similar analytical data have been obtained from other igneous and sub-volcanic complexes in this west Kenyan–east Ugandan–north Tanzanian Tertiary magmatic province. It seems that the apatites from any one particular rock type and texture all have the same chemical composition, no matter which igneous complex they are taken from in the province. Apatites, like other igneous minerals, seem to reflect chemically the nature of the liquids from which they were precipitated.

The five rock types mentioned under Table 1 provide good representatives of the ijolitic and the carbonatitic differentiation

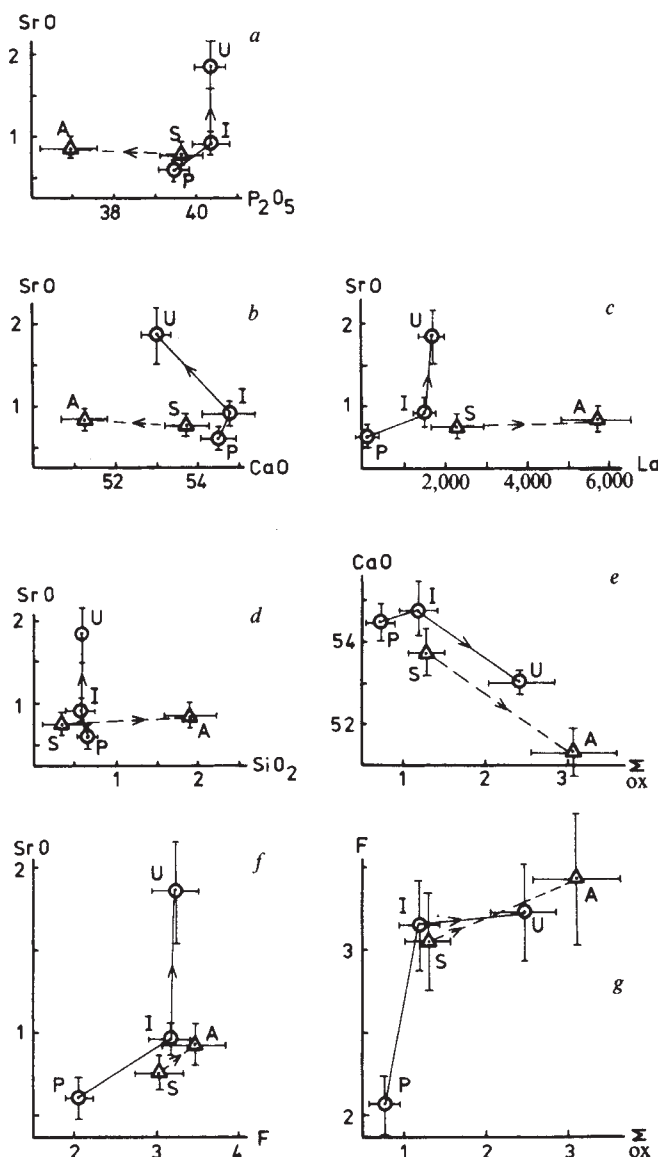


Fig. 1 Variation diagram for apatites from ijolitic (—○—) and carbonatitic (---△---) rocks. Oxides and fluorine plotted in wt %: La in p.p.m. Σ_{ox} is sum of Na, Ce, La, Sr oxides. Arrows show direction of differentiation. P, pyroxenite; I, ijolite; U, urtite; S, sövite; A, alvikite.

Table 1 EMP analyses of apatites (means)

	1 (n = 20)		2 (n = 33)		3 (n = 5)		4 (n = 30)		5 (n = 24)	
	wt %	s.d.	wt %	s.d.	wt %	s.d.	wt %	s.d.	wt %	s.d.
SiO ₂	0.65	0.08	0.56	0.18	0.60	0.05	0.30	0.15	1.91	0.37
P ₂ O ₅	39.46	0.30	40.43	0.50	40.38	0.29	39.75	0.55	36.97	0.70
CaO	54.50	0.41	54.84	0.64	53.00	0.16	53.78	0.55	51.34	0.50
SrO	0.61	0.10	0.89	0.13	1.85	0.37	0.74	0.09	0.83	0.11
Ce ₂ O ₃	0.03	0.03	0.12	0.02	0.35	0.04	0.21	0.06	1.49	0.20
La ₂ O ₃	0.01	0.01	0.17	0.02	0.20	0.02	0.27	0.07	0.67	0.09
Na ₂ O	0.08	0.03	0.04	0.06	0.04	0.01	0.10	0.03	0.10	0.15
F	2.07	0.19	3.16	0.27	3.23	0.29	3.06	0.32	3.42	0.41
	97.41		100.21		99.65		98.21		96.73	
less O = F	0.87		1.33		1.36		1.29		1.44	
	96.54		98.88		98.29		96.92		95.29	

Analyses were carried out on a Cambridge Mark V Electron Micro-Probe Analyser at 15 kV and specimen current of 20 nA and counts obtained were corrected for dead-time and ZAF using a modified MAGIC IV program.

1 From alkali pyroxenite, Usaki, West Kenya (U 1122).

2 From ijolite, Usaki, West Kenya (U 1256).

3 From urtite, Usaki, West Kenya (U 79).

4 From sövite, North Ruri, West Kenya (N 43).

5 From alvikite, North Ruri, West Kenya (N 179).

series. The pyroxenite is a product from an earlier partly cumulative stage in the ijolitic differentiation series than is represented by ijolite. The ijolite (comprising mainly nepheline and aegirine-augite) is the approximate plutonic equivalent of a nephelinitic magma, whilst the urtite (largely composed of nepheline) is the fractionation product. These three represent a typical sequence of the ijolitic magmatic differentiation series as recognised at most ijolite intrusive complexes^{1,3}. The sövite is the earliest and main member of the carbonatite series, and the alvikite is the second member of the series derived from the sövite by magmatic differentiation^{1,7}.

The structural formulae of the five mean apatite analyses (Table 2) are calculated on the basis of 10 ions in the calcium position⁸ rather than to 26 anions. This allows an estimate to be made, by difference, of the carbonate content of the apatite, based on the assumption that CO₃ groups substitute for PO₄ groups in the lattice⁹.

The substitution inferred of P by Si in notable amounts is real because most of the apatite crystals analysed from silicate rocks were single crystals mounted in resin, and that the apatite crystals were checked for lack of inclusions such as sometimes exist in these crystals¹⁰. The increasing proportion of Si with differentiation in the carbonatitic apatites is in keeping with the fact that free silica is commonly developed in late-stage carbonatites¹. The reliability of the estimate of the hydroxyl content is less certain, although the F determinations in Table 1 have a good precision and Cl was below the detection limit (100 p.p.m.). The elements Mg, Fe and Mn were also sought but were below the minimum detection limit (all near 200 p.p.m.), as was Ba (500 p.p.m.).

The distinctions between the silicate and carbonate differentiation series, which are reflected in the chemical variations of the apatites, are best seen in Fig. 1. Figure 1a-d show the divergence of the two trends, *e* and *g* show that grouping of some elements produces parallel trends but still shows the proposed common parentage, and *f* shows that the apatites from the silicate rocks can exhibit more extreme fractionation than those from the carbonatites: a situation which could not arise if the carbonatites were a fractionational crystallisation product of the ijolitic magma. Two further significant features of all the plots in Fig. 1 are that both trends have a common starting position, and that this position is near that for ijolite and sövite which are themselves considered to be the sub-volcanic materials most closely related to the parental compositions of the two differentiation series.

The close similarity in composition of the apatites from both ijolite and sövite shows that the relationship between the two liquids from which these two rocks crystallised could have been that of a pair of liquids in equilibrium with each other. It has already been suggested that the ijolitic and carbonatitic rock series are related by liquid immiscibility^{1,11} but, as far as we know, this is the first time that liquid immiscibility has been shown to be possible in natural alkaline igneous rocks based on the occurrence of one mineral phase, apatite, being in equilibrium with two liquid phases. This test for liquid immiscibility was recognised by Bowen¹² when he stated "Not only are they (the immiscible pair of liquids) in equilibrium with each other but they both must be in equilibrium with any additional phase".

The variable distribution of major and minor elements in apatites within ijolitic and carbonatitic alkaline igneous rocks

Table 2 Ions on basis 10 (Ca, REE, Sr, Na) for apatite analyses in Table 1

	1	2	3	4	5
Si	0.11	0.09	0.10	0.05	0.34
P	5.67	5.76	5.87	5.76	5.53
Ca	9.92	9.89	9.77	9.87	9.74
Sr	0.06	0.09	0.19	0.07	0.09
Ce	0.00	0.00	0.02	0.01	0.10
La	0.00	0.01	0.01	0.02	0.04
Na	0.02	0.01	0.01	0.03	0.03
F	1.11	1.68	1.76	1.65	1.91
(CO ₃)	0.22	0.15	0.03	0.19	0.13
(OH)	0.89	0.32	0.24	0.35	0.09
(O, OH, F)	26.39	26.58	26.88	26.52	26.56

show that their respective differentiation trends follow two independent paths, both emanating from magmatic compositions which could have been in equilibrium with each other. This relationship is consistent with liquid immiscibility rather than crystal fractionation as the governing petrogenetic process.

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First results from the Reykjanes Ridge Iceland Seismic Project 1977

PALAEOGEOGRAPHIC reconstructions of the North Atlantic area, based on plate tectonic principles¹⁻³, suggest that Iceland is of pure oceanic origin as Greenland and Europe (including Rockall Plateau) seem to have been closely connected before 56 Myr BP (magnetic lineation 24). Oceanic origin is also suggested by the location of Iceland astride the axis of the Mid-Atlantic Ridge, by its surface geology and by the young age (16 Myr) of its oldest rocks. Further evidence comes from refraction seismic investigations⁴ yielding a layering of the Icelandic crust resembling the typical oceanic crust in velocity values, but with greater thickness of individual layers. A remarkable result was the discovery of a layer with *P*-wave velocities close to 7.2 km s⁻¹ at a depth of 8–15 km, which was interpreted as the top of an anomalous upper mantle below Iceland. According to studies of teleseismic travel time residuals^{5,6}, as well as apparent velocity measurements of *P*-arrivals from Mid-Atlantic Ridge earthquakes⁷, this anomalous mantle may extend to 240 km depth. A very different model, however, has been derived from more recent long range refraction seismic measurements⁸. It is characterised by a more or less continental deeper crust and a normal upper mantle with a *P*-wave velocity of 8.0 km s⁻¹ at a depth of 50–60 km below Iceland. This model could also account for the low apparent velocities given by Francis⁷ and, if true, would require drastic modifications of current ideas of seafloor spreading. It has been used as an argument against the possibility of any significant amount of drift at the latitude of Iceland⁹. Geophysical evidence from Reykjanes and Kolbeinsey Ridges^{10,11}, on the other hand, strongly favours an oceanic origin of the surroundings of Iceland. Earlier refraction seismic measurements on the crest and western flank of the Reykjanes Ridge^{12,13} indicate a systematic change of crustal structure with age and a crustal structure typical of oceanic basins has been found some 200 km south-east of the ridge¹⁴. All the marine refraction work so far has shown anomalously low mantle velocities (7.2–7.8 km s⁻¹) but very little information is available on the structure of the oceanic upper mantle itself because of the limited range of the seismic

profiles. In 1977 an 800-km long seismic refraction profile was shot across Iceland and along the southeastern flank of the Reykjanes Ridge (Fig. 1). The main purpose of the experiment was to resolve the structure of the crust and upper mantle to greater depth than previously possible and to study the transition from the oceanic to the Icelandic structure. This experiment was planned and carried out by an international working group (Reykjanes Ridge Iceland Seismic Project, RRISP).

The experiment described here was a joint land-sea project with a number of shotpoints on sea and land giving a set of reversed and overlapping profile segments and large observational distances. Figure 1 shows positions of shots and receivers. Nineteen shots with charges from 0.4 to 4.0 tons were fired and recorded on land by up to 42 mobile stations of MARS-66 type¹⁵ from Germany, 12 automatic stations from the Soviet Union and 35 direct recording stations of the Icelandic short period seismic network. The shots at A to F were recorded by the MARS-66 stations on the main line running from Heimaey at the southern coast mostly through the neovolcanic zone towards the north-east. Shots G and H were observed along the southeastern coast to give a reference profile in the older part of Iceland. In the marine part ocean bottom seismographs¹⁶ and anchored telemetric buoy systems with ground hydrophones¹⁷ recorded series of smaller shots (50–250 kg). The shots were spaced 1.8 km apart and could be recorded to 150 km range. In addition to the 800-km long main line observed 110 km east of the ridge crest three more refraction lines were observed at sea, one parallel and closer to the ridge axis and two perpendicular to it. Most of the marine profiles were covered also by sparker, gravity, total magnetic field and narrow beam echo sounder measurements.

We shall concentrate on the results obtained on the main line. Figures 2 and 3 are examples of a marine and a land profile segment respectively. The upper part of Fig. 2 shows the record section (reduction velocity is 6 km s⁻¹) obtained by shooting into the bottom hydrophone of buoy AB1 from the south-west. First arrivals can be fitted by three straight lines indicating a well defined layering of the crust. From this and the reversed line we obtain the model shown in the lower part of Fig. 2. Apart from small dips of the sea bottom and basement the layers are horizontal. The velocities and layer thicknesses are within the range of typical oceanic values. It is not known whether the velocity of 7.7 km s⁻¹ represents anomalous mantle or crustal layer 3b. From the records of OBS BI01, however, which show first arrivals with apparent velocities of 8.3 km s⁻¹ at distances greater than 90 km, it is evident that normal upper mantle is present at greater depth.

The upper part of Fig. 3 shows a typical land record section. It was obtained for shotpoint E and reduced by 7 km s⁻¹. The record sections for shots D, F, G and H look very similar. There are distinct differences in travel time and apparent velocity within the first 100 km reflecting crustal heterogeneity but apparent velocities at greater distances are rather uniform and close to 7.0 km s⁻¹. Clear first arrivals can be traced up to distances of 300 km, whereas later arrivals are difficult to correlate. Wave groups with apparent velocities around 8 km s⁻¹ which would indicate a normal upper mantle below Iceland can not be found. This holds also for records of the more distant shots A and B. In spite of charges of 4 and 2 tons, fired from RV Meteor in optimum water depth, only weak first arrivals were obtained from these shots at the recording sites in Iceland. As far as correlation of these arrivals is possible, again apparent velocities only slightly greater than 7.0 km s⁻¹ are obtained. On the other hand, if apparent velocities between shotpoints A, B and C are calculated for individual stations in Iceland one obtains values as high as 8.4 or even 9.0 km s⁻¹. This is further evidence that, contrary to Iceland, the 10 Myr old Reykjanes Ridge on the seaward continuation of the profile has a well developed lithosphere. From the records of shot C, which give apparent velocities close to 8.0 km s⁻¹ for some stations near the south coast but 7.0 km s⁻¹ for more distant stations, it becomes evident that the transition from the normal lithosphere of the

Reykjanes Ridge to the anomalous deep structure of Iceland takes place near the southern shelf edge. The lower part of Fig. 3 shows our latest model with the corresponding ray paths for shotpoint E as calculated by a ray tracing method¹⁸. The calculated travel time curve has been superimposed on the record section and fits the observed arrivals well. Although the evaluation is still in progress and modifications of the model are to be expected, we feel confident with the conclusion that the P-wave velocity of 7.0 km s^{-1} is reached below Iceland in 10–15 km depth. There is no room for a 30-km thick layer with velocities from 6.5 to 7.0 km s^{-1} which could be interpreted as continental. In depths below 10–15 km the velocity increases only slightly

and velocity reversals cannot be ruled out. This anomalous layer must extend to depths greater than 60 km, as a normal upper mantle at 50–60 km depth⁸ would produce travel time segments with apparent velocities close to 8 km s^{-1} , which have not been observed in the record sections.

On the other hand, it is known from surface wave studies¹⁹ that the top of the asthenosphere is to be expected at about 60 km depth below the flank of the Reykjanes Ridge. It seems therefore most natural to interpret the anomalous layer below Iceland as a diapiric updoming of the asthenosphere and the term 'anomalous mantle' seems quite adequate. Further evidence for this interpretation comes from the observation of

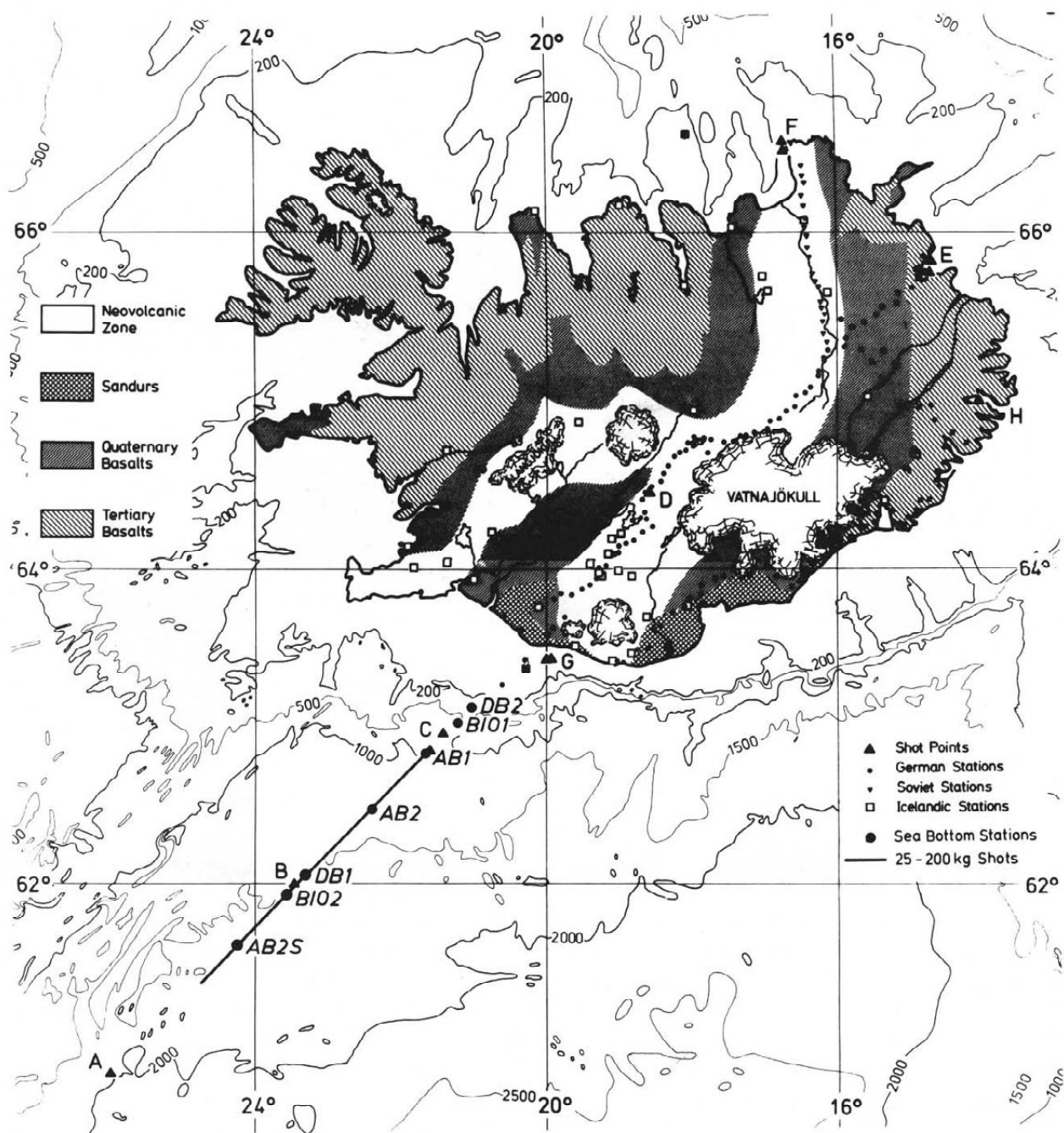


Fig. 1 Simplified geological map of Iceland and bathymetric chart of the surrounding ocean showing shotpoints and recording sites of the RRISP 77 project.

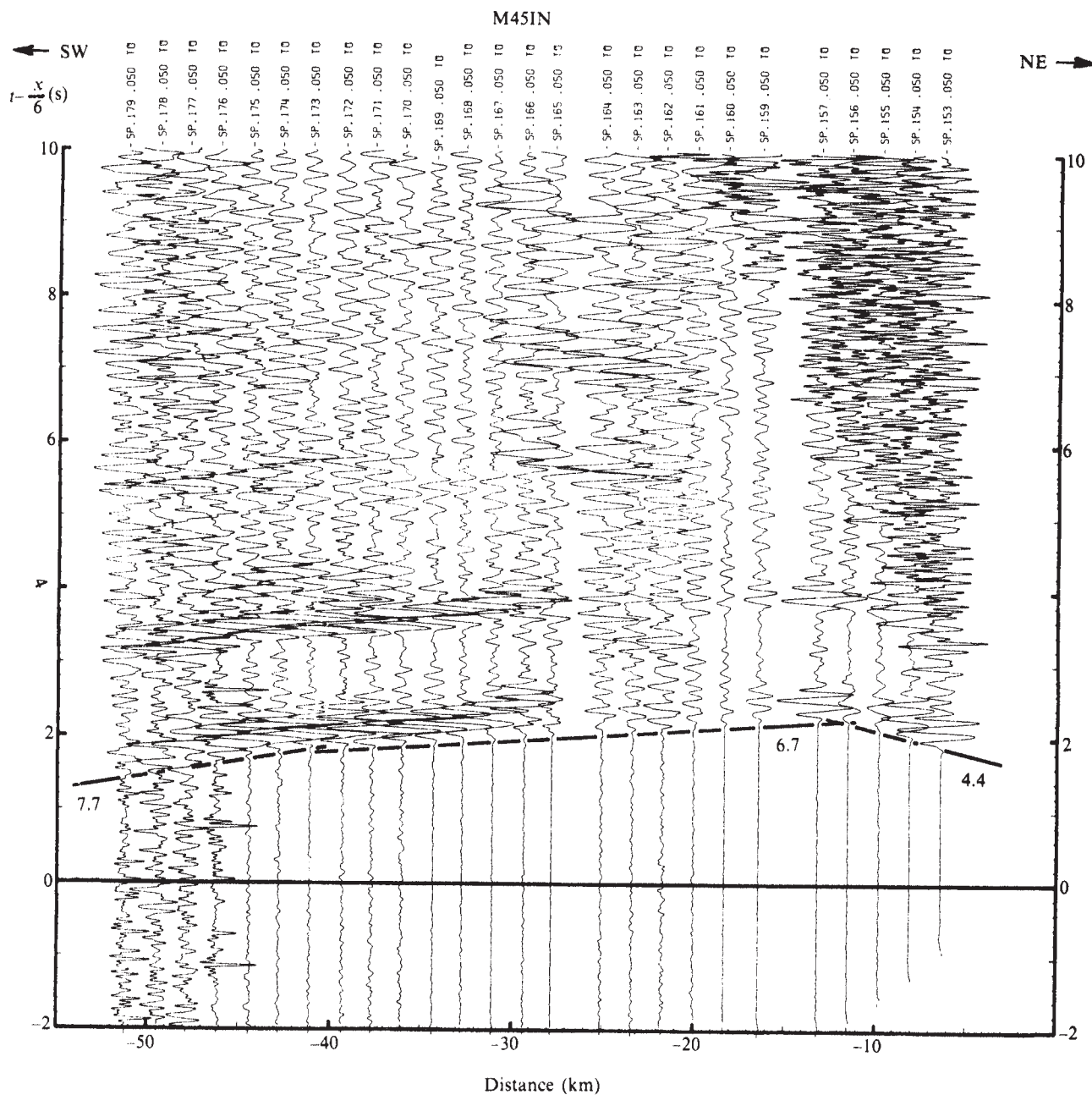


Fig. 2 Record section of the line AB1-AB2 recorded at the ground hydrophone of buoy AB1 and reduced by 6 km s⁻¹. The travel time curves are the ones calculated for the model in the lower part.

shear waves. The evaluation of both P- and S-waves, gives a normal ratio (1.76) for the P- to S-wave velocity in the uppermost 10–15 km, but a highly anomalous value (2.0) at greater depth, which may indicate a partially molten state of the anomalous mantle. This is also supported by magnetotelluric data²⁰.

In conclusion, Iceland is not simply an uplifted part of the Mid-Atlantic Ridge, but rather a deep-seated anomaly. Yet it

looks much more like an extremely active part than an obstacle to seafloor spreading in the North Atlantic.

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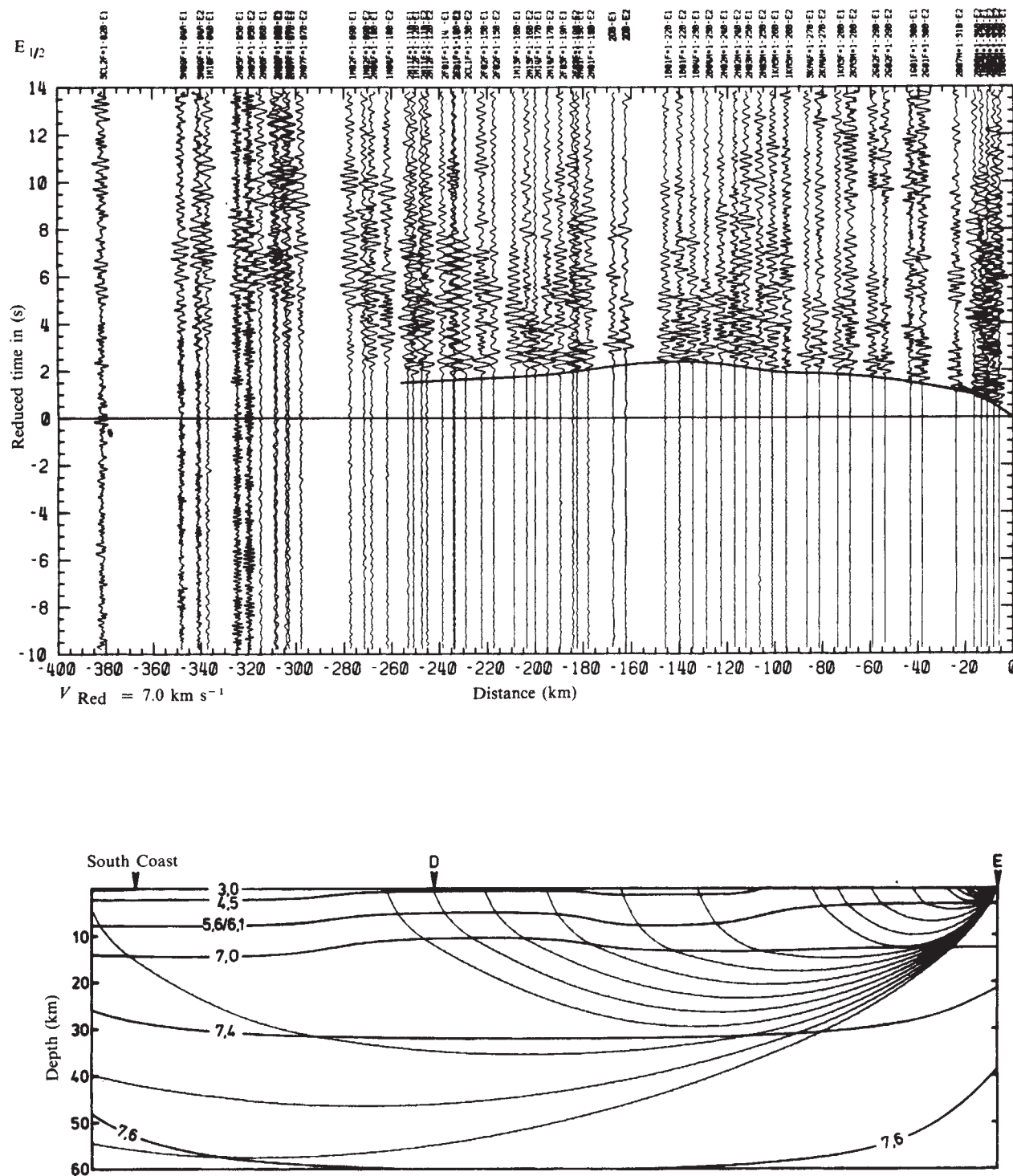


Fig. 3 Record section on the main RRISP line from shotpoint E towards south-west, reduced by 7 km s⁻¹. The travel time curve is the one calculated for the model in the lower part and only few of the calculated rays are shown. The model is defined by lines of equal velocity, indicated by the numbers inserted, and by linear vertical interpolation between neighbouring isolines. The depth range below the 7.0 km s⁻¹ isoline is interpreted as anomalous mantle.

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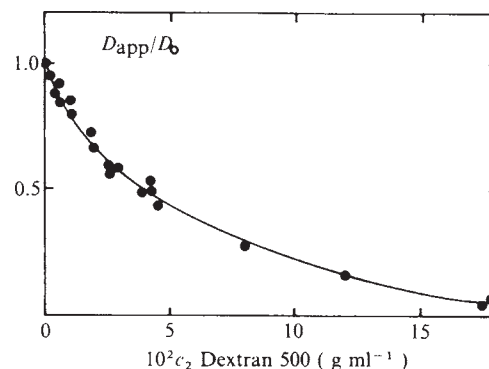


Fig. 1 Reduced diffusion coefficient (D_{app}/D_0) of bovine serum albumin (BSA) (component 1) as a function of the concentration of Dextran T 500 (component 2; molecular weight 5×10^5). D_0 is the diffusion coefficient of BSA in the absence of added dextran (B. N. P. and J. D. Wells, unpublished observations).

systems is therefore essential. Although problems of this type have been treated in a few theoretical^{1–7} and experimental^{8–20} studies, the understanding of these systems is incomplete, especially for concentrated solutions. It is known from studies of diffusion in binary systems that a number of features of the transport process appear that do not show up in more dilute systems^{7,10,18–20}. We present here new data for macromolecular systems which show that the diffusion rate of one macromolecule can be 'paradoxically' increased considerably by adding a sufficient amount of a suitable second macromolecular component. An interpretation of this effect is advanced in terms of an extension of existing theories of concentrated solutions.

The following discussion will be restricted to one homogenous phase, ignoring, for the moment, transport across phase boundaries such as membranes. The results apply, however, to transport inside a living cell or in the region between cells or inside a membrane.

The rate of diffusion transport^{1,21} (diffusion flow, J) in a binary system (solute + solvent) is determined by the value of the diffusion coefficient, D , and by the gradient in solute concentration, c , according to the relationship

$$J = -D \text{ grad } c \quad (1)$$

For a given concentration gradient (in living matter a steady state situation is often prevalent) the flow rate is determined by the numerical value of D . This coefficient depends on the thermodynamic as well as the hydrodynamic properties of the system according to the expression^{1,7,21}

$$D(c) = \frac{k \cdot T}{f(c)} \cdot Q(c) \quad (2)$$

where $Q(c)$ is a thermodynamic quantity, essentially the derivative of the virial expansion for the osmotic pressure, and $f(c)$ the frictional coefficient, a hydrodynamic parameter which is a measure of the flow resistance exerted by the medium on the solute. k is the Boltzmann constant, and T absolute temperature. When concentration increases both the 'thermodynamic factor' $Q(c)$ and the 'hydrodynamic factor' $f(c)$ usually increase and it is the interplay between these two quantities that determines the concentration dependence of D . The numerical values of Q and f and their functional form in terms of c depend on solute-solvent system, on the size and conformational properties of the macromolecular solute as well as on temperature and pressure. For macromolecules, Q is often the dominant factor and both the diffusion coefficient and the thermodynamic driving force may change by powers of 10 when the concentration is increased from infinite dilution to, say, 10% by weight¹⁸.

In multicomponent systems the situation is more complex due to the coupling of flows and the possibility of thermodynamic

Transport of molecules in concentrated systems

TRANSPORT of molecules in concentrated systems is of fundamental importance in medicine, biology and technology, for example, transport within cartilaginous tissues, the release and distribution within the body of encapsulated drugs and transport of reactants in the production process of plastic materials. These systems are usually multicomponent in nature. In all these processes the transport of substances often has a regulatory role as a rate determining step. An understanding of the transport process of macromolecules in multicomponent

interaction between solutes²². In a ternary system (solute 1 + solute 2 + solvent), for instance, there are two independent flows, J_1 and J_2 , and four diffusion coefficients related through the Onsager reciprocal relationship, thus leaving three of them as independent quantities. However, if in such a ternary system the concentration of solute 1 is low and if the interest is focused on how the rate of transport of this solute is affected by successive additions of solute 2, expressions of the general form (1) and (2) can still be used²⁴ as good approximations of reality. D is then to be interpreted as an apparent diffusion coefficient, D_{app} , governing the flow of component 1.

D_{app} depends on both the thermodynamic and the hydrodynamic interaction between solutes. Although there is a lack of systematic studies of thermodynamic interactions, certain osmotic pressure and light scattering data²³⁻²⁵ obtained for ternary systems indicate that in the case of two different chain polymers the interaction is strong (large excluded volume effects) and increases considerably with increasing concentration of one or both of the solutes (successively higher order

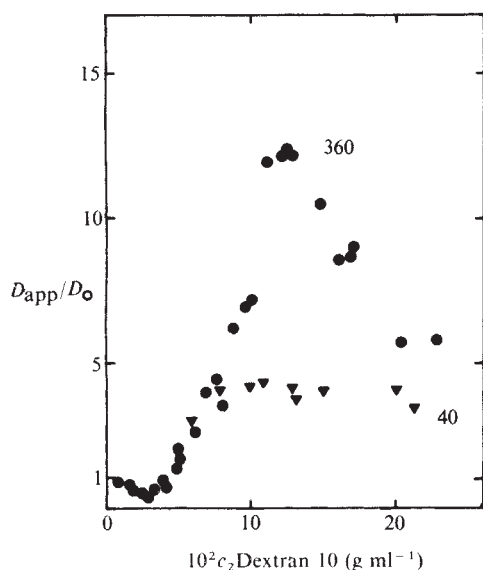


Fig. 2 Reduced diffusion coefficient (D_{app}/D_0) of polyvinylpyrrolidone (PVP) (component 1) as a function of the concentration of Dextran T 10 (component 2; $MW \approx 10 \times 10^4$). ●, PVP 360 ($M_n \approx 3.2 \times 10^5$); ▼, PVP 40 ($M_n \approx 4 \times 10^4$). (B. N. P. and R. G. Kitchen, unpublished observation).

terms in the viral expansion gain importance). The frictional properties of multicomponent macromolecular systems are less known. From some sedimentation and diffusion results^{4,5,9,12,14,16,24,26} and general knowledge about binary systems one may conclude, however, that the frictional coefficient will increase markedly on addition of the second solute, but less so for intermediate concentrations than does the thermodynamic factor (see below).

Recent experiments¹⁷ on the diffusion of one macromolecule (component 1) in dilute form in a solution containing increasing amounts of a second macromolecule (component 2) up to very high concentrations have revealed the following features. If component 1 is a compact globular particle (for example, bovine serum albumin (BSA)) diffusing in a solution containing dextran (component 2), a smooth decrease in D_{app} is observed (Fig. 1). These results are in agreement with earlier work^{9,14}. However, if both solutes are chain polymers, then a marked difference in behaviour is observed (Fig. 2). In this case, the diffusion rate of polyvinylpyrrolidone (PVP) first decreases with increasing c_2 ,

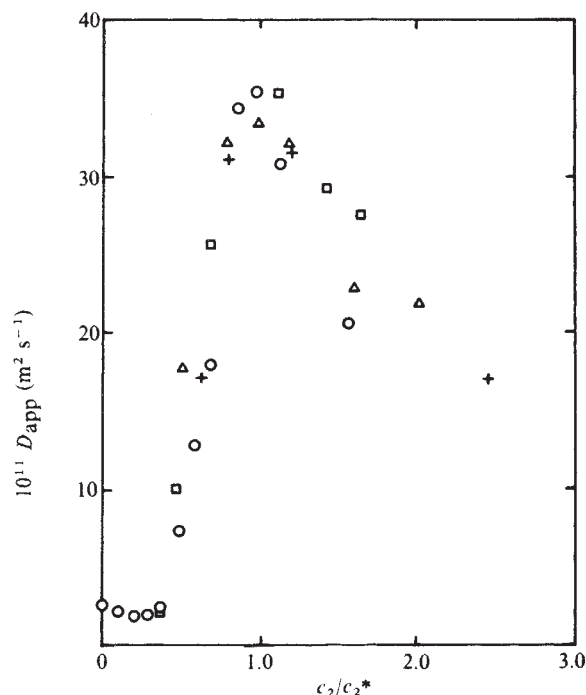


Fig. 3 Variation in D_{app} of PVP ($M_n \approx 3.2 \times 10^5$) (component 1) as a function of c_2/c_2^* for various dextrans (component 2) of different molecular weight. ○, Dextran T 10 ($MW \approx 1.0 \times 10^4$); □, Dextran T 20 ($MW \approx 2.0 \times 10^4$); △, Dextran T 70 ($MW \approx 7.0 \times 10^4$); +, Dextran T 150 ($MW \approx 15.0 \times 10^4$). c_2^* is calculated using a modified version²⁹ of a relationship as given by Simha and Zakin²⁸. (B. N. P. and G. Checkly, unpublished observations).

then increases markedly; D_{app} can reach very high values sometimes of the order of 45 times the value in the absence of component 2. Then D_{app} reaches a maximum and decreases at still higher values of c_2 . These results point out the regulatory influence of the second solute on the diffusion rate of the first.

These results seem to be in accord with the general view of diffusion in multicomponent macromolecular systems as discussed above, with the following qualitative explanation. The diffusion rate is governed by an interplay between 'thermodynamic' and 'hydrodynamic' factors. Component 1 is present in high dilution and, up to a certain but fairly low value of c_2 , the addition of component 2 mainly affects frictional properties and the diffusion rate of component 1 is lowered. When c_2 has been raised sufficiently the thermodynamic interactions between the different macromolecules will, in the case of the linear polymers, rapidly become very strong due to large excluded volume effects and will dominate over the increase in friction. This explains the very steep increase in the D_{app} curve against c_2 . Continued addition of component 2 finally gives a situation where close packing of the encompassed-volume-spheres of the solute molecules occurs. For dense hexagonal close packing, it has been suggested that this occurs at a concentration (c_2^0) given by $c_2^0 = 1.08/[\eta]$ where $[\eta]$ is the intrinsic viscosity of solute 2 (ref. 28). At this concentration, there will be a drastic increase in the 'friction' which eventually will dominate over the thermodynamic interactions.

If the molecular weight and hence molecular domain of component 2 in the system studied in Fig. 2 is increased, then the resultant D_{app} against c_2 curve is displaced to lower concentrations. However, if a normalised concentration scale is used, c_2/c_2^* , where c_2^* differs from c_2^0 only by a small factor that allows for the compression of solute 2 (B. N. P. and A. G. Ogston, in preparation), then the curves D_{app} against c_2/c_2^* become superimposable (Fig. 3). It appears that the reduced scale defines approximately corresponding physical states for the

different molecular weight species. It is pertinent that the maximum value of D_{app} occurs at the point of incipient overlap of the molecular domains of component 2 (that is $c_2/c_2^* = 1$).

That the molecular conformation of the diffusing species is critical in determining the diffusion features has been clearly demonstrated by study of the simultaneous diffusion of PVP and BSA in a concentrated solution of dextran. Whereas the diffusion transport of the chain polymer was enhanced by a factor of 12, that of albumin was reduced by a factor of 2 (unpublished observation).

This small note precludes a detailed theoretical examination of multicomponent diffusion and thermodynamic interaction but a number of features predicted by theory have been observed in the system discussed above. These include the marked dependence of D_{app} on: (1) molecular weight of component 1 (Fig. 2); (2) the concentration of both polymeric components; and (3) time.

Our observations may be relevant for understanding the rate and direction of diffusion in extracellular compartments containing high concentrations of, for example, polysaccharides or mucins. However, they may also be important for intracellular processes such as axonal flow.

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Ear ossicle of *Australopithecus robustus*

WE report here the discovery of the first ear ossicle, an incus, of a Plio-Pleistocene hominid. It is substantially different from that of modern man, and the dissimilarity exceeds that between the ear bones of *Homo sapiens* and of the African apes. The new incus is of interest particularly in view of the unique advantages that ear ossicles have for taxonomic and phylogenetic studies. (The only other fossil hominid ear ossicles are from Qafzeh¹ and are indistinguishable from those of modern man.)

The right incus was discovered in the *Australopithecus robustus* specimen SK 848, a temporal bone from Swartkrans (a distinct genus, *Paranthropus*, is recognised by one of us, R.J.C.). The incus was found anatomically *in situ*—in the epitympanic recess—and is complete except for the long process (Fig. 1). The near-perfect state of preservation permits detailed examination of the bone's morphology, including the articular surface.

The body of the Swartkrans incus is inflated and bulb shaped. A deep indentation at the centre of the articular surface emphasises the protrusion of the oversized dens, thus providing the body with a somewhat more symmetrical appearance than in man, gorilla and chimpanzee. The short (posterior) process is cylindrical and of small diameter. The area of its attachment to the body is narrow compared with the corresponding area in man and the African apes, where the process is flat and broadens anteriorly to meet the body. In *A. robustus* the small diameter of the process results in a concave superior contour and the elevation of the body above the process.

Comparison of the articular surfaces also reveals profound differences. The synovial incudomalleolar joint in man is usually described as a saddle-shaped biaxial joint, and in this respect it resembles the chimpanzee and the gorilla. The saddle shape enables the superior part of the articular facet to be exposed in medial view (Fig. 1*b, c, d*, top) and the inferior part (on the dens) to be exposed in lateral view (Fig. 1*b, c, d*, middle). This twisting of the surface from the medial to the lateral sides of the bone is not found in *A. robustus*, where a view of the medial side does not include the articular surface (Fig. 1*a*, top). The two parts of the articular surface (superior and inferior) face each other, forming a more uniaxial joint shaped like the semilunar notch of the ulna. Only in *A. robustus* does the superior part of the articular surface occupy such a small portion of the bone's body (Fig. 1*a*, bottom).

Although the incus is tiny, its importance should not be overlooked. Furthermore, this is one of the most complete and undistorted bones of *A. robustus* yet discovered. The study of its morphology with respect to taxonomy and phylogeny is aided by additional advantages unique to this bone. It is fully formed at birth, and thus later changes are minimal. It has no muscles anchored on it and so exhibits none of the morphological effects often associated with muscular attachment. As Hershkovitz (ref. 2, p. 176) states in reference to the ear ossicles, "Their growth patterns, size, shape, position, and composition are not significantly influenced or modified by movements or stresses generated by the growth or remodeling of unrelated neighboring bones. In effect, ontogeny of the ossicle is virtually a complete and undisturbed expression of the basic controlling genetic factors. Importance of ear bone morphology and ontogeny in phylogenetic reconstructions cannot be underestimated." This is more true of the incus than the malleus^{3–5}.

Specialisations of the ear are usually found in structures other than the ossicles, such as the pinna and the external auditory meatus, the tympanic bulla, the fenestra ovalis and the ligaments supporting the ossicles; specialisations are also exhibited by certain anatomical relationships, such as the proportion between the areas of the tympanic membrane and the fenestra ovalis^{3–5}. Many of the modifications associated with the ear ossicles themselves are related to the position and orientation of

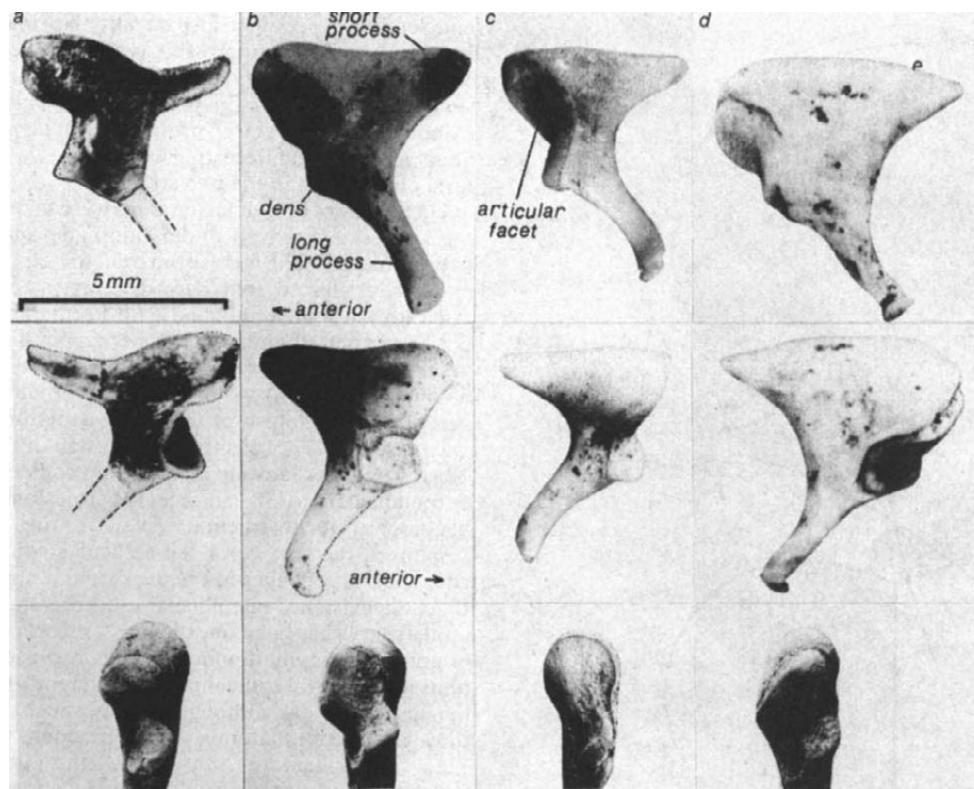


Fig. 1 Comparison of the right incus of *A. robustus* (a) with that of modern man (b), chimpanzee (c) and gorilla (d). Top row, medial view of the bone; middle row, lateral view; bottom row, view of the articular surface. All bones are on the same scale and orientation.

these bones in the tympanic cavity and to the elongation of their processes³⁻⁵. Furthermore, the malleus—the most external ossicle in the chain—tends to be more specialised than the incus²⁻⁵. Only in cases of extreme modification of the ear, as in bats, moles and whales, is the morphology of the incus substantially affected³⁻⁵. It is interesting that the acute angulation of the articular facet's surface, which distinguishes the *A. robustus* incus from that of the African apes and man, is also characteristic of the rodent *Dipodomys merriami*, whose ear is specialised to an unusually low frequency range³. Furthermore, the extremely delicate short process in *A. robustus* (the site of attachment of the posterior ligament connecting the incus to the petrous bone) may indicate that, as in *D. merriami*, the ossicles were loosely suspended in the tympanic cavity³.

The solid appearance of the SK 848 incus and of its articular surface suggests that its shape is not the result of some pathological process. Its unusual morphology is far beyond the range of normal variation characteristic of the incudes of modern man and the great apes⁴. This is also supported by the observations made by one of us (Y.R.) on tens of *H. sapiens* bones from different populations and on five gorilla and 10 chimpanzee bones.

Although the specific phylogenetic and taxonomic implications of our findings are unclear because of the lack of a comparative fossil incus sample, this bone can provide at least a notion of how great a phylogenetic deviation is represented by the robust australopithecines.

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Changes in the uncrossed retinotectal projection after removal of the other eye at birth

THE connections between the eyes and the brain are so arranged as to give an orderly representation in the brain, of the visual field. The mechanisms involved in this ordering have been extensively studied, particularly in the retinotectal pathway of submammalian vertebrates¹⁻³. In these species the optic nerves decussate almost completely, retinal ganglion cells in one eye connecting only with the opposite tectum. In mammals, however, there is partial decussation at the optic chiasma, so that the superior colliculus receives optic fibres from both eyes. Binocular representation poses a problem for the ordering of retinotectal projections. If one point on the tectum is to correspond to only one point in visual space, seen through both eyes, the mapping rules onto the tectum must have opposing polarities in the two eyes, along the nasotemporal axis. This is shown in Fig. 1: a rostral movement in the colliculus corresponds

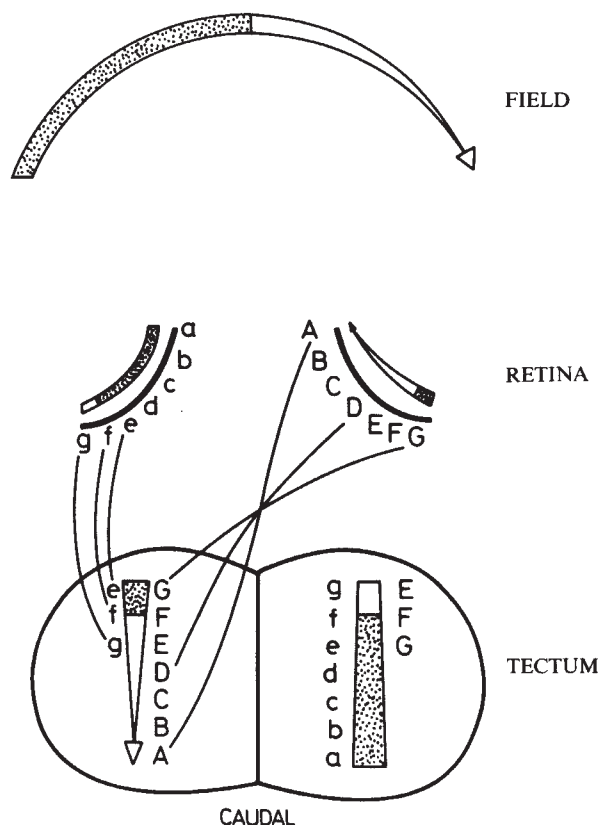


Fig. 1 A schematic diagram showing the representation of the visual field on the retina and on the tectum of a mammal such as the hamster. The horizontal meridian of the visual field is depicted as an arrow and is shown projecting onto the two tecta so that the central field is found rostrally and the peripheral field caudally. There are connections from the temporal retina of both eyes to the rostral portion of each tectum such that one point on the tectum corresponds to only one position in the central visual field, seen through both eyes. It is apparent that the ordering of the projection shows opposing nasotemporal polarities in the ipsilateral and contralateral retinas. In left tectum the sequence G F E from the contralateral eye is matched by e f g in the ipsilateral eye; in the right tectum g f e is matched by E F G.

to a temporal movement on the contralateral retina but a nasal movement on the ipsilateral retina. Little is known about the factors determining the topography of the direct ipsilateral projection to the mammalian superior colliculus. Anatomical studies in rodents have shown that neonatal removal of one eye induces an increased projection from the remaining eye to the ipsilateral colliculus^{4,5}. These aberrant uncrossed optic fibres are derived from all regions of the retina and apparently have a distribution appropriate for a normal contralateral projection, that is, the nasotemporal retinal axis is represented caudorostrally⁶. The only published physiological study investigating the topography (in enucleated rats) confirmed the anatomy as regards the nasotemporal axis but, surprisingly, found the dorsoventral axis reversed over most of the colliculus ipsilateral to the remaining eye⁷. Here I present results on the enucleated hamster showing that the colliculus ipsilateral to the remaining eye contains a double representation of the visual field displaying mirror-image polarity along the nasotemporal axis.

I have examined electrophysiologically the retinal representation in the superior colliculi of hamsters, *Mesocricetus auratus*, which had one eye removed at birth. I used both normally pigmented and albino strains in an attempt to distinguish the contributions to the retinotectal map of fibres which would normally have gone ipsilaterally, and of fibres induced by

enucleation, to project ipsilaterally. Normal albino mammals have only a small uncrossed retinal projection⁸ and I have confirmed this for the hamster using both anatomical and physiological techniques (in preparation). This difference in the extent of the ipsilateral projection in pigmented and albino hamsters makes it interesting to compare the effects of contralateral enucleation in the two strains.

On the day of birth hamster pups were taken from the nest and the right eye removed under fluothane anaesthesia. Animals over 3 months old were prepared for electrophysiology in a manner similar to that described by Tiao and Blakemore⁹. Anaesthesia was maintained with urethane-chloralose (25 mg per kg per h urethane; 0.16 mg per kg per h chloralose) and the animals were paralysed with gallamine triethiodide (Flaxedil, 35 mg per kg per h). After removing the dura the visual cortex was usually left intact, protected by a mixture of liquid paraffin and vaseline. The electrode (tungsten in glass¹⁰, tip length 12–18 μ m) was advanced into the colliculus approximately perpendicular to its surface; microlesions were made for histological reconstruction. Even in the absence of visual responses, the surface of the colliculus could be identified by characteristic changes in the background noise.

As the existence of collicular units driven through the ipsilateral eye^{11–13} has been questioned¹⁴, recordings were first made in normal pigmented animals. Cells recorded during penetrations in the rostral colliculus were frequently responsive through the ipsilateral eye although usually much more weakly than through the contralateral eye, in agreement with the findings of Tiao and Blakemore¹¹. In general, the part of the tectum in which there were ipsilaterally evoked responses agreed with the distribution of uncrossed optic terminals defined by anatomical methods (in preparation). In addition, receptive fields in the two eyes always shifted together in visual space as the electrode was moved across the colliculus. Consequently, the situation shown in Fig. 1 does represent that of the normal hamster.

Recordings were made in 12 enucleated animals (six albino and six normally pigmented): 299 penetrations into the mid-brain ipsilateral to the remaining eye and 50 in the opposite side (two animals). Multi-unit activity was recorded as soon as the electrode entered the colliculus and the receptive field was plotted by moving spots of light on a hemispherical screen in front of the animal. Receptive fields were generally 10–20° in diameter, occasionally much larger, but no double receptive fields were seen⁷. Compared with the visual responses from the ipsilateral eye in the normal animal, those in the enucleated animal were much brisker and were recorded over a far greater extent of tectum.

Figure 2 shows typical collicular maps of the visual field for animals enucleated at birth and recorded when adult. A comparison of the uncrossed and crossed projections in one animal (Fig. 2, WH 115/1/3) shows that there is a reversal within the retinotectal topography of the uncrossed projection. Whereas a caudorostral movement in the colliculus contralateral to the remaining eye gives a normal monotonic progression from temporal to nasal visual fields, such a movement in the ipsilateral colliculus corresponds initially to a temporonasal field progression, which then reverses to move from nasal to temporal fields. The polarity of the retinotopic map in the rostral tectum is appropriate for a normal ipsilateral projection whereas that in the caudal tectum is appropriate for a projection to the contralateral side.

This pattern of ipsilateral retinotectal topography following removal of one eye at birth, was seen in all 10 animals in which the mapping was sufficiently extensive. There is no obvious difference between pigmented and albino animals (Fig. 2). The retina maps continuously onto the tectum but with two polarities; consequently, parts of the retina are represented in two places on the tectum. It is only the central part of the visual field (temporal retina) that is mapped twice. As in the normal animal, the rostral colliculus represents only the central (normally binocular) visual field. The aberrant projection to the caudal colliculus includes both central and peripheral visual field and is

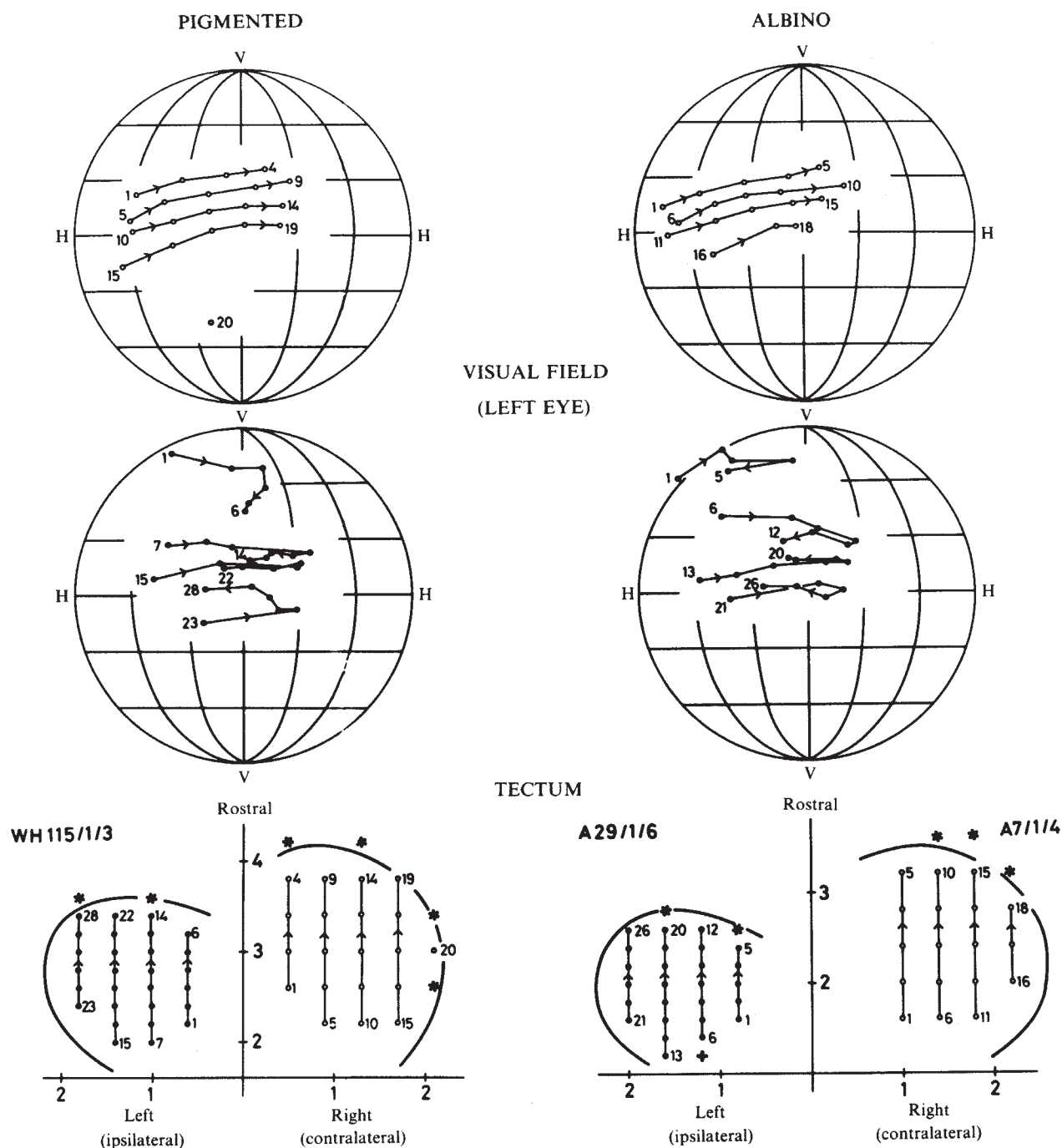


Fig. 2 The mapping of the visual field, seen through the left eye, onto the superior colliculi, is shown for the pigmented hamster (left) and the albino (right). The right eye of each hamster had been removed on the day of birth. Filled circles indicate the locations of penetrations into the left colliculus (ipsilateral to the remaining eye) and also the position of the corresponding receptive fields in visual space; contralateral penetrations and the matching receptive fields are depicted by open circles. The electrode, which was angled 35° forward from the vertical in the parasagittal plane, was moved in a regular matrix of penetrations over a horizontal stereotaxic plane through lambda. The axes (in mm) at the bottom of the figure plot the intersections of the penetrations with this plane, the origin being the lambda. The points representing each parasagittal line of penetrations are joined and the progression from caudal to rostral tectum is indicated by the arrowheads. Asterisks denote penetrations falling outside the colliculus and the cross refers to one penetration within the colliculus on which no visual response could be elicited. Above the collicular representations are the visual field maps. In each case, the upper map (open circles) is derived from penetrations in the contralateral (right) colliculus and the lower (filled circles) from ipsilateral penetrations. The visual field coordinate system is that used by Tiao and Blakemore¹¹, using axis-vertical coordinates for the hemisphere whose anterior pole lies directly in front of the animal. The meridians and parallels are shown at 30° intervals; H-H and V-V are the horizontal and vertical meridians. To compensate for divergence of the eyes under relaxant, a small correction was applied to the positions of the receptive fields, assuming that the normal projection of the optic disk (monitored with an ophthalmoscope) lies 54° from the vertical meridian and 25° above the horizontal meridian¹¹.

consequently compressed compared with the normal crossed projection.

Thus, after removal of one eye at birth, the uncrossed fibres exhibit retinotectal mapping polarities appropriate for both contralateral and ipsilateral projections in the normal animal. This implies that the mechanisms involved in determining these polarities are independent of whether or not decussation occurs and that the map in the rostral colliculus, appropriate for a normal ipsilateral projection, does not require the presence of an intact projection from the opposite eye. A further surprising feature is the segregation of the two maps in the ipsilateral tectum. The aberrant input showing mapping rules appropriate to a contralateral projection might have been expected to fill the whole colliculus, with the two maps superimposed in the rostral half as they are in the normal animal (where they come from different eyes).

This segregation could arise if the aberrant axons, induced to project ipsilaterally by enucleation of the other eye but exhibiting contralateral mapping rules, arrive at the tectum much later than would a normal crossed projection and find themselves excluded from the rostral tectum because of proliferation of the normal ipsilateral termination in that region. However, this argument might predict that the area of rostral tectum occupied by a normal ipsilateral map should still be very small in the enucleated albino; surprisingly, it is not (Fig. 2). Thus, in albinos at least, some of the fibres that are induced to project ipsilaterally, nevertheless, behave appropriately for a normal ipsilateral projection; perhaps these are the fibres that the albino mutation would normally cause to pass contralaterally rather than ipsilaterally¹⁵⁻¹⁷.

An important question is whether the physiologically determined retinotectal map actually reflects the pattern of direct retinal ganglion cell input^{18,19}. In this context further anatomical work is necessary to ascertain whether the apparent discrepancy between the maps found in this study and those determined anatomically in the rat⁶ is real. A further complication is the existence in mammals of a projection from the visual cortex to the superior colliculus^{20,21}, the removal of which, in the cat, decreases ipsilateral driving of tectal neurones²². Cunningham and Speas⁷ removed the cortex before recording, but it is unlikely that this explains the disparity between their results and mine, as I found that removal of the visual cortex, in two animals, had no significant effect on the topography of the ipsilateral projection mapped before and after the lesion. The topography was also unaffected by subsequent removal of the colliculus contralateral to the remaining eye.

These findings imply that the normal direct projection from the eye to the ipsilateral tectum (occurring to a significant extent only in mammals) has an inherent mapping specificity which is independent of input from the other eye. The observation that two maps of different polarity, arising from one eye, can co-exist within a single tectum may mean that a single area of retina contains two populations of ganglion cells exhibiting opposed polarity labels. Alternatively, it could be that the caudal and rostral halves of the tectum provide different mapping cues for fibres entering from the ipsilateral eye. A further complication arises if uncrossed fibres in both normal and enucleated animals are branches of crossed fibres, as has been suggested for the rat^{23,24}. In that case, the two branches of a single retinal ganglion cell would have to display different mapping rules according to their laterality. Further work is required to provide fundamental information about the formation of visual maps in the mammalian brain.

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Blood platelets do not provide endoperoxides for vascular prostacyclin production

PROSTACYCLIN (PGI₂), the most active natural inhibitor of blood platelet aggregation described so far, is synthesised from the cyclic endoperoxide, PGH₂, derived from endogenous arachidonic acid by the cyclo-oxygenase enzyme system. Exogenous endoperoxides are also readily converted into PGI₂ (refs 1, 2). Indomethacin inhibits the cyclo-oxygenase activity and thereby prevents PGH₂ formation. Because of this lack of precursor, endogenous PGI₂ production of indomethacin-treated tissue does not occur. However, when vascular tissue, pretreated with indomethacin, is incubated in platelet-rich plasma (PRP), its PGI₂ production is restored. On the basis of this observation it has been suggested that activated blood platelets can be a source of exogenous endoperoxides, stimulating the vascular prostacyclin formation and, consequently, limiting the growth of a platelet thrombus³. We now report results indicating that it is highly unlikely that blood platelets are able to promote vascular prostacyclin formation by supplying cyclic endoperoxides.

Male Wistar rats, specific pathogen-free, mean body weight about 300 g, were bled under ether anaesthesia by puncturing the abdominal aorta. The blood was collected in sodium citrate (3.8 w/v % 1 part + 9 parts of blood) and PRP and platelet-poor plasma (PPP) were prepared by differential centrifugation. Aortas were removed, cleaned from adjacent tissue, opened longitudinally and kept in Krebs-Henseleit buffer with or without indomethacin (2 µg ml⁻¹) for at least 60 min at 0 °C. A small piece of tissue (diameter 3 mm, dry weight ~100 µg) was punched out of the aorta and incubated immediately in 200 µl of either PPP or PRP in a gently shaken plastic vial for 3 min at room temperature. Measurement of the prostacyclin content of the incubate was based on the inhibiting effect of the incubate on ADP-induced aggregation of blood platelets. Incubation of normal aortic tissue resulted in the production of an aggregation-inhibiting principle with typical prostacyclin characteristics⁴. No significant difference in PGI₂ production was observed on incubation with either PPP or PRP (Fig. 1).

Indomethacin-treated tissue did not produce prostacyclin, irrespective of whether it had been incubated in PPP or PRP (Fig. 1). Similar results were obtained with longer incubation times (up to 12 min) and/or using human PPP and PRP as incubation media. Prostacyclin formation of normal aortas was considerably stimulated and that of indomethacin-treated aortas completely restored when PGH₂ was added to the

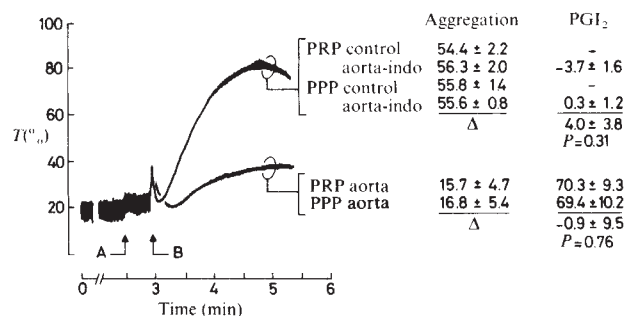


Fig. 1 Prostacyclin production of rat aortic tissue, some treated with indomethacin (aorta-indo), on incubation (3 min) in platelet-poor rat plasma (PPP, platelet count $8-10 \times 10^3 \mu\text{l}^{-1}$) or platelet-rich rat plasma (PRP, platelet count $1.7 \times 10^6 \mu\text{l}^{-1}$) at room temperature. The incubate (see text) was centrifuged (45 s; Beckman 152 microfuge) and 1 min after termination of incubation 50 μl was added (A) to an aggregometer cuvette (Born/Michel) containing 0.75 ml saline (0.85 w/v % NaCl, 7 parts + CaCl_2 $4 \times 10^{-3} \text{mol l}^{-1}$, 1 part) and 0.1 ml rat PRP, which had been preincubated (37 °C) for 2.5 min. Thirty seconds after incubate addition 0.1 ml ADP was added (B) to a final concentration of $2.5 \times 10^{-7} \text{mol l}^{-1}$. Aggregation is indicated by the % change in light transmission (ΔT). Prostacyclin content of the incubates was calculated from the difference in aggregation in the presence of aorta-containing incubates (b) compared with aorta-free incubates (a) and expressed as % inhibition: $\text{Inh. \%} = (a - b/a)100\%$. Mean values $\pm$ s.e.m. of seven determinations.

incubation medium ($1 \mu\text{g ml}^{-1}$ final concentration), thus showing the responsiveness of the tissue to exogenous precursor in the present experimental conditions. Therefore, it can be concluded that PRP, gently shaken at room temperature for 3 min, does not supply the vascular wall with endoperoxides for prostacyclin synthesis.

Because this PRP treatment might not have activated the platelets sufficiently to initiate arachidonate peroxidation, in the next series of experiments platelet endoperoxide production

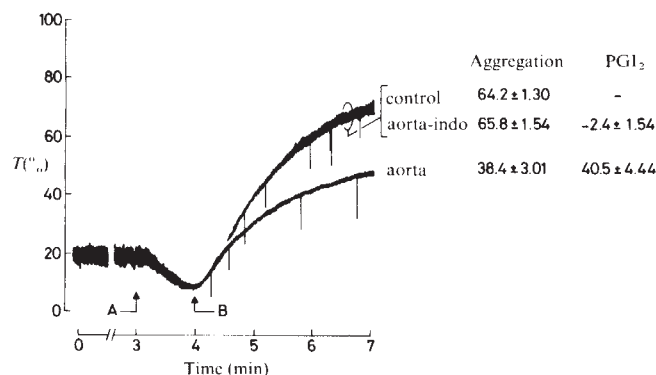


Fig. 2 Prostacyclin production of rat aortic tissue which had (aorta-indo) or had not (aorta) been preincubated (≥ 60 min) in indomethacin-containing Krebs-Henseleit buffer ($2 \mu\text{g ml}^{-1}$). Aggregation of rat PRP (platelet count $1.7 \times 10^6 \mu\text{l}^{-1}$) was triggered (A) by collagen ($5.6 \times 10^{-6} \text{g protein ml}^{-1}$ final concentration). At the beginning of aggregation (delay time ~ 60 s) aortic tissue was added (B). Aggregation was quantified as the % change in transmission 3 min after adding the tissue (ΔT). Prostacyclin production was determined by measuring the difference in collagen-induced aggregation after tissue addition (b) compared with control aggregations without added tissue (a) and expressed as % inhibition: $\text{Inh. \%} = (a - b/a)100\%$. Mean values $\pm$ s.e.m. of 16 determinations.

was triggered by collagen. PGI₂ formation by normal and indomethacin-treated aortic tissue was assessed on incubation in PRP during aggregation induced by a collagen dose that caused platelets to produce endoperoxides (measured as malondialdehyde) and to release part of their granule content⁵. The results (Fig. 2) show that indomethacin-treated aortic tissue is unable to produce prostacyclin even if it is incubated in a suspension of collagen-activated blood platelets.

We then investigated whether the prostacyclin production of vascular tissue not treated with indomethacin could be influenced by incubation in PRP producing different amounts of endoperoxides. For this, we used platelets and aortas of normal rats and animals deficient in essential fatty acids (EFA). Platelets of these latter animals, when triggered by collagen, aggregate less than normal platelets^{6,7} and produce only small amounts of endoperoxides and thromboxanes^{5,7,8}. Their vascular prostacyclin production is lower than that in normal animals⁴, but thromboxane⁷ and prostacyclin synthetase⁴ are present in normal amounts.

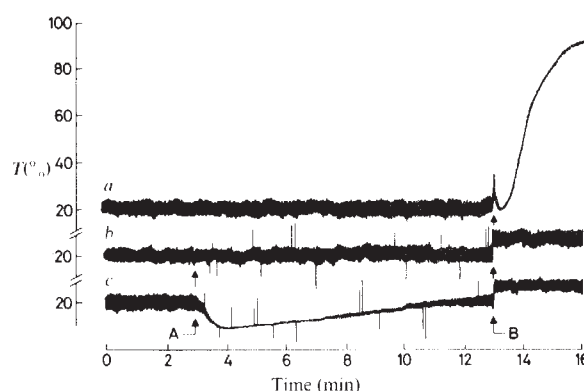


Fig. 3 Effect of normal and indomethacin-treated aortic tissue on the % light transmission (T) of PRP and ADP-induced aggregation in PRP. A PRP dilution (for details see legend to Fig. 1) was stirred (1,000 r.p.m.) in an aggregometer at 37 °C. After 3 min (A) a piece of aorta was added. Ten minutes later (B) the tissue was removed and ADP added to trigger aggregation. *a*, No aorta. Aggregation indicates response of platelets to ADP. *b*, Normal aorta. No platelet shape change and aggregation after either tissue addition or ADP administration. In later experiments this was shown to be the result of prostacyclin formation by the added tissue. *c*, Indomethacin-treated aorta. The immediate decrease in transmission and the diminishing oscillations indicate activation of the platelets by the vascular tissue. After some time the tracing tended to return to normal, indicating de-activation of the platelets. Together with the absence of aggregation on ADP addition, this suggests progressively increasing prostacyclin formation, which was confirmed in later experiments (not shown).

If platelet endoperoxides stimulate vascular prostacyclin formation, one would expect more PGI₂ to be formed on incubation of vascular tissue with collagen-activated normal than EFA-deficient PRP. However, Table 1 shows that neither normal nor EFA-deficient aortic tissue produce more PGI₂ on incubation with collagen-activated normal PRP than with collagen-activated EFA-deficient PRP.

Because normal as well as EFA-deficient vascular tissue can easily be stimulated to produce more PGI₂ merely by adding exogenous PGH₂ to the incubation medium, it is highly unlikely that activated blood platelets contribute to vascular prostacyclin synthesis by providing cyclic endoperoxides. As shown by Bunting *et al.*³ and confirmed by others^{9,10}, including ourselves (Fig. 3), indomethacin-treated tissue prevents aggregation when incubated in PRP. The mechanism for this is unknown, but we

Table 1 Prostacyclin production of rat aortic tissue on incubation with collagen-aggregated PRP of normal or EFA-deficient rats

Aorta	PRP	Prostacyclin (ng PGE ₁ per min)	Difference
Control	Control	9.1 ± 1.32	
Control	EFA-deficient	7.8 ± 0.91	$P > 0.10$
EFA-deficient	Control	1.3 ± 0.58	$P < 0.001$
EFA-deficient	EFA-deficient	1.4 ± 0.41	$P > 0.10$

For technical details see the legend to Fig. 2. As the collagen-induced aggregation is depressed in EFA deficiency^{6,7}, effects were calibrated against the inhibitory effect of prostaglandin E₁ (PGE₁). Dose-response curves of PGE₁ were made in both normal and EFA-deficient PRP. Prostacyclin production is given in PGE₁ equivalents (ng min⁻¹), mean ± s.e.m. of 11 determinations. Results were statistically evaluated using the Kruskal-Wallis analysis of variance on ranked observations¹¹.

believe that it is due to removal of indomethacin from the cyclo-oxygenase enzyme system, which is consequently reactivated during incubation. This was confirmed by the following experiment.

A piece of indomethacin-treated rat aorta was added to a stirred rat PRP dilution and the change in transmission recorded continuously. When after 10 min the tissue was removed and ADP ($0.1 \text{ ml}, 2.5 \times 10^{-6} \text{ mol l}^{-1}$) added to the PRP, no aggregation was observed (Fig. 3). Aggregation did occur, however, when the platelet mixture was stirred without added aorta (Fig. 3), confirming Bunting's observation that indomethacin-treated vascular tissue produces PGI₂-like activity when incubated in stirred PRP.

The removed tissue was quickly rinsed in Krebs-Henseleit buffer, incubated in 200 µl Tris buffer (0.02 mol l^{-1} , pH 7.2) at

room temperature, and endogenous PGI₂ production assessed in 50-µl portions of the incubate taken after 3, 10 and 20 min of incubation. If the cyclo-oxygenase was still blocked by indomethacin, no prostacyclin production would be expected. However, substantial amounts of endogenous PGI₂-like activity were detected in the incubate (Fig. 4). This is not due to 'late' conversion of stored, platelet-derived PGH₂, as essentially similar results were obtained on 10 min incubation of indomethacin-treated tissue in a stirred PPP dilution or in a dilution of Krebs-Henseleit buffer (Fig. 4). Endogenous PGI₂ production could be prevented only when the first as well as the second incubation was carried out in a saline milieu, containing indomethacin (Fig. 4).

Indomethacin added to PRP or PPP ($2 \mu\text{g ml}^{-1}$) did not prevent reactivation of vascular cyclo-oxygenase (not shown), most probably because the added indomethacin is bound to plasma proteins, resulting in too low a 'free' indomethacin concentration. There was no appreciable difference between the use of PRP or PPP during the first incubation on subsequent endogenous PGI₂ production by the tissue. This indicates that platelets do not have a special effect on reactivation of vessel wall cyclo-oxygenase. In this respect, plasma seems superior to buffer, probably because the strong affinity of indomethacin for plasma proteins facilitates its removal from vascular cyclo-oxygenase.

In our first experiment (Fig. 1), we were unable to detect PGI₂ production by indomethacin-treated aortas incubated in PRP or PPP. Obviously, the incubation conditions in our last experiment (temperature 37°C instead of room temperature; time 10 instead of 3 min; plasma mixing by stirring instead of shaking) enhanced the removal of indomethacin from vessel wall cyclo-oxygenase.

Later experiments, to be presented elsewhere, showed that reactivation of indomethacin-blocked vascular cyclo-oxygenase needs about 6 min of incubation in plasma at 37°C , stirred at 1,000 r.p.m. The presence of platelets during this incubation had no significant effect.

Thus, we have shown that in a variety of conditions blood platelets do not enhance vascular prostacyclin production by supplying cyclic endoperoxides. Experiments suggestive of the existence of such a pathway did not take into account that the binding of indomethacin to cyclo-oxygenase, and consequently, the cyclo-oxygenase inactivation is reversible and can easily be overcome by incubation of indomethacin-treated tissue in an indomethacin-free medium.

Note added in proof: Recently, Needleman *et al.*¹² reached a similar conclusion, namely that in normal conditions blood vessels do not use platelet cyclic endoperoxides for prostacyclin synthesis.

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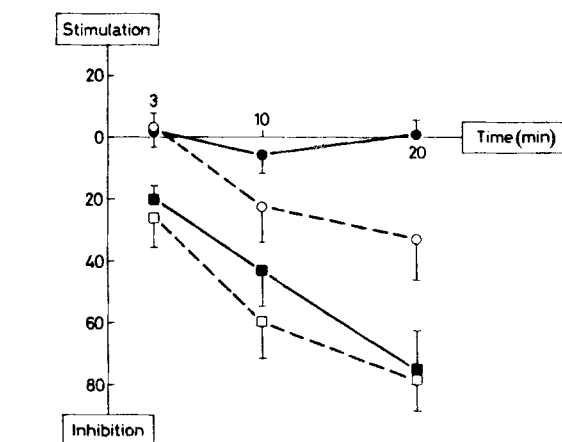


Fig. 4 Endogenous prostacyclin production (% inhibition of ADP-induced aggregation) of indomethacin-treated aortic tissue of rats after preincubation. Pieces of vascular tissue were added to an aggregometer cuvette containing 0.8 ml saline + CaCl₂ (see Fig. 1) and 0.1 ml PRP, PPP or Krebs-Henseleit buffer. This mixture had been preincubated for 3 min at 37°C , while stirred at 1,000 r.p.m. After 10 min the tissue was removed, rinsed in Krebs-Henseleit buffer and incubated in 200 µl Tris buffer (0.02 mol l^{-1} , pH 7.2) at room temperature in a gently shaken vial. After 3, 10 and 20 min 50-µl portions of the incubate were assessed for their PGI₂-like activity on the basis of their inhibiting effect on ADP-induced aggregation (see Fig. 1). Results are means ± s.e.m. of seven determinations. First and second media were, respectively: ■, PRP, Tris; □, PPP, Tris; ○, Krebs-Henseleit, Tris; ●, Krebs-Henseleit + indomethacin ($2 \mu\text{g ml}^{-1}$ final concentration), Tris + indomethacin ($2 \mu\text{g ml}^{-1}$).

Is alanine the active component in anterior pituitary extracts proposed to contain a thymotropic factor?

SAXENA AND TALWAR have reported the occurrence of a factor in extracts of the anterior pituitary which stimulates thymidine incorporation into thymocytes *in vitro*¹. The possible existence of a previously unknown thymotropic pituitary factor was discussed. The test system used by Saxena and Talwar consisted of isolated rat thymocytes cultured in a defined glucose salt medium without serum or other additives. Anterior pituitary extract (APE) was added and the uptake of ³H-thymidine into acid-insoluble material during short-term incubation was greatly stimulated. It was briefly mentioned that stimulation was also obtained when thymocytes were cultured in RPMI or Parker 199 medium. No effect was observed on tissue slices from liver, heart, kidney and diaphragm. The factor was said to have a molecular weight of 500 and an isoelectric point of 7.9, but the methods of determining these properties were not described. We report here on the isolation of an active component in the APE and its identification as the amino acid alanine. APE was tested for the ability to stimulate thymidine incorporation and mitotic activity in guinea pig thymocytes in our *in vitro* systems (test systems are described in the legends to Table 1 and Fig. 1). The incorporation of thymidine and the mitotic activity were both stimulated in our experiments (Fig. 1a). The stimulatory effect on thymidine incorporation was dose dependent and varied between 129 and 156% in different experiments.

APE was purified using preparative thin layer chromatography (TLC). In the first system (cellulose; ethanol 95%: ammonium acetate 1M, pH 5.0; 7:3), at least 10 separate ninhydrin-positive bands were obtained. Fractions were eluted according to the ninhydrin stain and tested for the ability to stimulate thymidine incorporation. Active material was rechromatographed in other solvent systems, fractionated and again tested for activity. A fraction which was considered homogeneous was on analytical TLC found to correspond to the amino acid alanine. When alanine was used as a reference in the preparative runs, all the activity was found in the region of this amino acid. (Amino acids mentioned are in the L form unless indicated otherwise.)

The RPMI 1640 medium, used by us and many others for lymphoid cell cultures, contains all common amino acids except alanine and cysteine². It has recently been discovered that alanine, when added to RPMI 1640 medium, causes a quantitatively important increase in the thymidine incorporation into thymocytes and lymphocytes of different organs and species³. Isolated rat liver cells are not stimulated by alanine (O.S., unpublished results). The addition of alanine also increases the frequency of mitoses in cultures of guinea pig thymocytes (Fig. 1b and ref. 4). The stimulatory effect of alanine in RPMI 1640 medium is detected at a concentration of 10 μ M and is maximal at 100 μ M. Concentrations up to 2 mM cause the same maximal stimulation. D-Alanine or β -alanine do not stimulate thymidine incorporation. Alanine is one of the most common amino acids in serum⁵ and the alanine requirement would probably be provided for by the addition of serum to the culture medium. However, uncontrolled factors such as general enzyme inhibitors and uncharacterised growth factors present in serum render its use unsuitable in studies of lymphoid growth factors.

Lymphoid cells from different species seem to vary in sensitivity to alanine stimulation. In guinea pigs, for example, thymocytes are stimulated by less than 200%, whereas human thymocytes could be stimulated by more than 350% in RPMI 1640 medium (O.S., unpublished results).

Saxena and Talwar¹ used a defined glucose salt medium for their thymocyte cultures. We studied the effect of supplementing such a medium with nutrients, such as amino acids and vitamins. Table 1 compares the radioactivity incorporated into

thymocytes cultured in RPMI 1640 medium, in RPMI 1640 supplemented with alanine, and in Hank's-Dulbecco (H-D) medium (a mixture of equal parts of Hank's balanced salt solution and Dulbecco's phosphate-buffered saline, the compositions of which are described in ref. 2). The highest incorporation is found in the alanine-supplemented RPMI 1640 medium and the lowest in the H-D medium. Table 1 also shows the effect of addition of APE or alanine to the different media. When APE was tested for activity in alanine-supplemented RPMI 1640 medium, no stimulation of the thymidine incorporation was obtained. Thus, the stimulatory effect of APE in the RPMI 1640 medium depends on its alanine content. Material in extracts from bovine liver, spleen and thymus, which stimulated the thymidine incorporation into lymphocytes from various sources, has earlier been identified as alanine³. As shown in Table 1, addition of alanine is without stimulatory effect on thymidine incorporation in the H-D medium, indicating that the stimulatory effect of APE in this medium depends on alanine in combination with other amino acids, probably present in the extract. (Crude low molecular weight material (<10,000 daltons) from bovine thymus stimulates thymidine incorporation into guinea pig thymocytes cultured in H-D medium (O.S., unpublished data). Even highly purified low molecular weight material from tissue extracts (thymus, spleen and liver) contains, in addition to alanine, various amounts of other amino acids (K. Nordlind, personal communication).

We conclude that the stimulatory effect of APE on thymidine incorporation and the frequency of mitoses in RPMI 1640

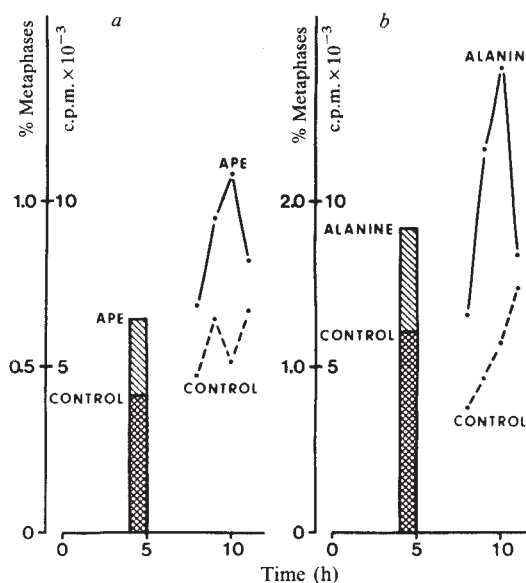


Fig. 1 Effects of APE (a) and alanine (b) on incorporation of ³H-thymidine and frequency of colcemid-arrested metaphases in cultured thymus cells. The method for determination of ³H-thymidine incorporation is described in the legend to Table 1. For quantification of mitotic activity, 1-ml cultures with 5×10^6 cells were incubated in RPMI 1640 medium at 37°C. After 30 min, APE (1:2; see Table 1) or alanine (to make 0.65 mM) was added (time 0) in a volume of 0.2 ml. The procedure for preparation of the cells for mitotic counts is described elsewhere⁴. Briefly, cells were swelled on glass slides by hypotonic treatment, fixed by addition of methanol:acetic acid (3:1) and stained; 5,000 cells from each group were examined at $\times 1,000$ magnification. Incorporation of ³H-thymidine (c.p.m.) in 1 h is shown by vertical bars, and mitotic activity (% metaphases) by continuous (APE or alanine) and dashed lines (controls). Each point on the curves represents the frequency of cells in metaphase after a 2-h pulse with colcemid (100 μ l added to make 0.08 μ g ml⁻¹). The values for ³H-thymidine incorporation and mitotic activity after addition of APE (but not alanine) were measured at the same time using cells from the same suspension in both cases.

Table 1 Thymidine incorporation into guinea pig thymocytes cultured in different media, and the effect of addition of APE or alanine

Addition	Incorporated radioactivity (c.p.m., mean of duplicate cultures)		
	RPMI 1640	RPMI 1640 + alanine (0.5 mM)	Hanks-Dulbecco
Control (sterile water)	7,602	12,446	4,033
Alanine (50 nmol per culture)	11,712 (154)	12,538 (101)	3,206 (79)
APE 1:2	10,381 (137)	10,274 (83)	5,530 (137)
1:4	9,905 (131)	11,114 (89)	5,294 (131)
1:6	8,505 (112)	10,861 (87)	4,771 (118)

5×10^5 guinea pig thymocytes were incubated in 0.1-ml samples of the media indicated above. Incubations were carried out in a Linbro micro-titration plate in 37°C in an atmosphere of 10% CO_2 in air. Penicillin (100 IU ml^{-1}) and streptomycin ($100 \mu\text{g ml}^{-1}$) were added to all media and glutamine (2 mM) was added to the RPMI media before the onset of cultures. One ampulla of lyophilised APE was dissolved in 5 ml sterile water (according to instructions from R. K. Saxena) and diluted as indicated above; $10 \mu\text{l}$ of APE or alanine was added to duplicate cultures from the start. Control cultures received $10 \mu\text{l}$ of sterile water. Tritiated thymidine ($0.5 \mu\text{Ci}$, $1 \mu\text{M}$) in $10 \mu\text{l}$ physiological saline was added after 4 h. One hour later the cultures were collected on a Skatron multiple cell collector using glass fibre filters. Incorporated radioactivity was measured in a Packard liquid scintillation spectrometer. Values in parentheses show the percentage of incorporation in the different media after the addition of alanine or APE compared with the control cultures.

medium are due to the presence of alanine in the APE. The effect of APE in glucose salt medium, shown by Saxena and Talwar, is probably caused by the presence of several essential nutrients in the APE, for example, a mixture of amino acids. As shown here, the growth of thymic cells (and probably other cells also) in short-term cultures is very dependent on the presence of essential nutrients. When culturing lymphoid cells, alanine should be included in the culture medium.

Due to the facts presented above, we feel that the existence of a new thymotrophic pituitary factor is not yet fully confirmed.

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Amputation of a suppressor determinant on lysozyme reveals underlying T-cell reactivity to other determinants

THE response to several thymus-dependent antigens is influenced by immune response (*Ir*) genes, usually those of the major histocompatibility complex. However, the relevant gene products and their cellular sites of action have not been fully characterised. It is evident that in certain cases, the *Ir* genes can

function to select those determinants which will be presented to activate T cells. This control operates, at least in part, at the level of macrophage-T-cell interactions. We have previously proposed¹ that the failure of a T-cell response in H-2^b mice following immunisation with chicken lysozyme (HEL) could be due to preferential stimulation of suppressor T cells. A suppressor mechanism which nullifies the activity of lysozyme-specific T-helper cells has been reported from our laboratory². Comparison of the amino acid sequence of various closely related lysozymes reveals that molecules which have tyrosine at position 3 are immunogenic in H-2^b mice whereas lysozymes with phenylalanine at position 3 are non-immunogenic in such strains³. We have considered the possibility that the latter lysozymes possess a determinant that preferentially stimulates suppressor cells in H-2^b mice, whereas the former lack this 'suppressor determinant'. Other studies⁴⁻⁶, including those by Schwartz *et al.*⁷ and Turkin and E.E.S.⁸, support a phenomenon of generalised suppression caused by restricted determinants. Here, we demonstrate that a strain which is unresponsive to HEL and whose T-cell proliferative activity is absent following immunisation with the entire molecule, possesses the potential to generate a vigorous T-cell response directed against a large fragment of the antigen. Suppressor cells directed against a restricted determinant on the molecule obscure this competence to respond to the majority of the molecule.

As a first test of the proposition that HEL carries a suppressor determinant, a major peptide of HEL lacking the putative

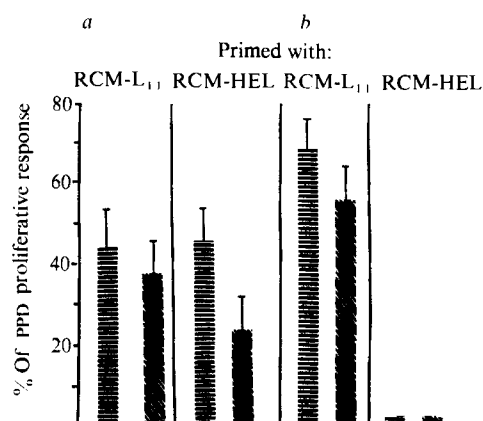


Fig. 1 Groups of 10 C57BL/10 (a) and 10 C57BL10.A (b) mice were injected with either $15 \mu\text{g}$ of RCM-L₁₁ or $22 \mu\text{g}$ RCM-HEL, emulsified in complete Freund's adjuvant (containing *Mycobacterium tuberculosis* strain H37Ra), divided in the hind footpads. Four weeks later, peritoneal exudate T-enriched lymphocytes (PETLES) were prepared as described by Schwartz *et al.*¹⁰ Five days after injection of 1 ml of 10% thioglycollate, the peritoneal cells were recovered, washed and passed over nylon wool to enrich for T cells¹¹. 2×10^5 PETLES per well were stimulated in round-bottomed microtitre plates (Microbiological Associates) with medium alone (modified Eagle-Hanks' medium containing fresh L-glutamine, 2-mercaptoethanol, antibiotics and 10% heat-inactivated fetal bovine serum), or supplemented with $50 \mu\text{g ml}^{-1}$ purified protein derivative (PPD), or antigens ($4\text{--}200 \mu\text{g ml}^{-1}$). Cultures were incubated at 37°C in 2% CO_2 , 98% air for 5 d. Four days after initiation, cultures were pulsed with $1 \mu\text{Ci}$ of [Methyl-³H]thymidine (specific activity 6.7 Ci mmol^{-1} , NEN) in $10 \mu\text{l}$ phosphate-buffered saline. The amount of antigen-stimulated incorporation of label into cellular DNA was determined by counting the cells collected 16 h later on filter disks in a liquid scintillation counter. The maximum stimulation observed in three separate experiments has been plotted as a relative per cent of the response stimulated by PPD (purified protein derivative of the same strain of *M. tuberculosis*). PPD stimulation ranged from 25–50,000 c.p.m. in different experiments; the background (medium alone) was 500–2,000 c.p.m. The bars represent mean incorporation $\pm$ s.e.m. The antigen used in culture was either RCM-L₁₁ (■) or RCM-HEL (▨).

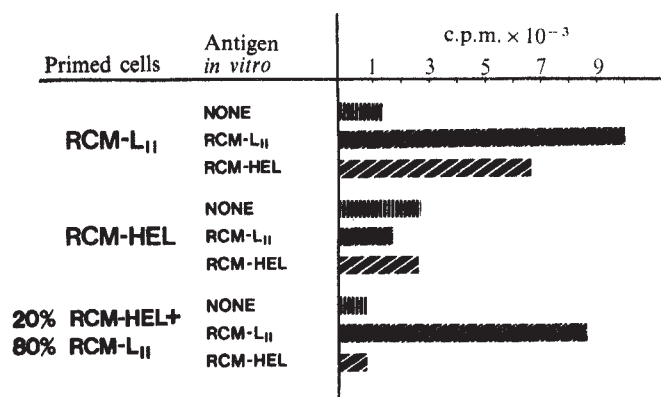


Fig. 2 Using an identical protocol to that described in the legend to Fig. 1, groups of B10 mice were primed with 15 μ g RCM-L_{II} or 22 μ g RCM-HEL, and the PETLES were isolated 4 weeks later. The individual or mixed PETLES populations were stimulated as shown, with 50 μ g ml⁻¹ RCM-L_{II} or 75 μ g ml⁻¹ RCM-HEL. Mixtures of 2%, 20% or 50% RCM-HEL-primed PETLES with RCM-L_{II}-primed PETLES were used, resulting in 22, 10 and 25% of the RCM-L_{II}-induced level of incorporation, respectively. A representative experiment is shown, in which only the 20% mixture results are plotted.

suppressor determinant was used to prime B10 and B10.A congeneric mice, and the *in vitro* proliferative potential of these peptide-primed T cells was determined. The peptide used was the reduced and carboxymethylated, cyanogen-bromide peptide RCM-L_{II} (amino acid residues 13–105). This is the largest peptide derivable from HEL which lacks the presumptively suppressive region around the N-terminus. As this peptide was obtained from RCM-HEL, one underlying assumption that had to be tested was that the T-cell cross-reactivity observed between HEL and RCM-HEL⁹ extended to the suppressor cell level. Therefore, groups of B10 and B10.A mice were immunised with intact RCM-HEL and the proliferative responses of T cells from these mice were determined in the peritoneal exudate T-enriched lymphocyte (PETLES) system first developed by Schwartz *et al.*¹⁰. Whereas RCM-HEL induced a vigorous PETLES proliferative activity in the B10.A strain, there was no response to RCM-HEL by B10 PETLES. We then tested the prediction that B10 mice should be capable of responding to RCM-HEL following 'amputation' of the suppressor-inducing determinant. B10 and B10.A mice were immunised with 15 μ g of RCM-L_{II} (a molar equivalent of the standard dose of intact RCM-HEL) in complete Freund's adjuvant. Three to six weeks later, PETLES were prepared and the proliferative response of these cells was determined. As shown in Fig. 1, both B10 and B10.A PETLES from mice primed with RCM-L_{II} demonstrate active proliferative responses to this large peptide. Furthermore, the stimulatory determinants recognised on RCM-L_{II} are also available on intact RCM-HEL, as shown by the high degree of cross-stimulation of RCM-L_{II}-primed PETLES in the B10.A case. In contrast, the distinct preference for RCM-L_{II} by B10 PETLES may reflect the effect of primary suppressor T-cell recognition of the suppressor determinant on intact RCM-HEL.

The functional inability of RCM-HEL-primed B10 PETLES to proliferate in the presence of strong responsiveness to determinants on RCM-L_{II} can be attributed to a suppressor-inducing determinant present on RCM-HEL, but absent from RCM-L_{II}. Taken together, the regions absent from RCM-L_{II} (residues 1–12 and 106–120) represent a determinant region very similar to the N–C disulphide peptide. This peptide has previously been shown to induce HEL-specific suppressor cells in B10 mice¹. The combined evidence supports the concept that there is a population of precursor T cells available in B10 mice which can be effectively stimulated by determinants on RCM-L_{II}.

The implication that RCM-HEL is able to raise suppressor T cells which can affect the proliferative response to RCM-L_{II} was then tested directly in the experiment shown in Fig. 2. RCM-L_{II}-primed PETLES, when stimulated by homologous peptide in culture, gave a proliferative response that was 55% of the value induced by purified protein derivative (PPD). RCM-HEL-primed PETLES again showed no proliferation *in vitro*, when stimulated by either RCM-HEL or RCM-L_{II}, suggesting that no expansion of RCM-L_{II}-specific T cells had occurred. These putatively suppressive cells were then titrated into the responsive, RCM-L_{II}-primed population. As shown in Fig. 2, 20% of the RCM-HEL-primed PETLES completely obliterate the specific response; actually, this was true even when the proportion of suppressive cells was only 2%. It is particularly interesting that the suppression only occurred over an antigenic bridge which linked the suppressor determinant with the proliferation-inducing determinant. Thus, when suppressor cells were present and potentially fully active, RCM-L_{II} added in culture stimulated a response similar to that seen in the absence of suppressor T cells. However, with RCM-HEL as antigen, suppression was easily demonstrable. This dominant suppressive effect obviated the possibility that for some reason, RCM-HEL was catabolised too quickly in B10 mice to make an impression on the immune machinery.

This reasonably active suppression on a primed T-cell proliferative response has previously been difficult to observe. It suggests that the most suitable system in which to detect suppression may be one, as above, in which the antigen (fragment) stimulating the target response, here RCM-L_{II}, does not itself act on suppressor cells.

We conclude that many situations arise in systems under obvious genetic control^{2,7}, but also with more complex antigens, where a particular haplotype will regard a determinant region as suppressive. The existence of this recognition will then obscure an underlying, vigorous responsiveness directed against other parts of the molecule.

One, if not the major role, of *Ir* genes may be performed by antigen-presenting cells (macrophages) in selecting the appropriate determinant to be offered to different subpopulations of T cells^{12,13}. Although this has widely been considered as a problem relating to the activation of helper cells, our results indicate that suppressor cell triggering is the critical factor for the outcome of the total response. Whether antigen presentation to suppressor cell precursors is a macrophage function remains to be established. Our work suggests that what occurs is the presentation of different determinants to the competing subpopulations of regulatory T cells.

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Solubilisation of high-affinity dopamine receptors

NEUROLEPTIC drugs are thought to act by blocking brain dopamine receptors¹. *In vitro* binding assays using either ³H-haloperidol^{2,3} or ³H-spiperone^{4,5} have provided more direct evidence of this interaction. Although spiperone has been reported to label serotonergic receptors in the frontal cortex⁶, in the rat striatum, in both *in vitro*⁴⁻⁹ and *in vivo* conditions^{5,10,11}, the binding characteristics of this drug are dopaminergic. Initial attempts to solubilise the neuroleptic receptor were recently undertaken in our laboratory, but the ³H-spiperone macromolecular complex obtained from rat striatal membranes after digitonin treatment¹² did not show the original high-affinity binding characteristics for dopamine agonists and antagonists except for spiperone itself. More recently, solubilised neuroleptic sites were found in digitonin extracts from calf caudate but they were still not characterised¹³. We now report the solubilisation of binding sites from dog striatum which retain the characteristics of membrane-bound dopamine receptors.

Mongrel dogs were anaesthetised with pentobarbital and Wistar rats were decapitated; their brains were removed and the striatum, cerebellum and frontal cortex were dissected out and homogenised in 0.25 M sucrose. A microsomal (P) fraction was prepared as previously described⁴. This membrane preparation was treated at 0 °C for 15 min with 1% digitonin suspended in 2 or 4 volumes of 0.25 M sucrose containing 10 mM sodium phosphate buffer (pH 7.2) and 0.01% NaN₃. After centrifugation at 120,000 g (*r*_{av}) for 60 min in a SW 65 Ti Spinco rotor, the supernatant, which was considered as the soluble preparation (~2 mg protein ml⁻¹), was removed very carefully without disturbing the pellet. An aliquot of this solubilised preparation was incubated at 0 °C for 16 h in the presence of 2 × 10⁻⁹ M ³H-spiperone (specific activity 23.6 Ci mmol⁻¹, NEN) and various concentrations of unlabelled drugs. Aliquots of 0.1 ml of the incubation mixture were layered on the top of a Sephadex G-50 Medium column (13 × 0.5 cm). Elution was carried out at 2 °C with 10 mM sodium phosphate (pH 7.2) containing 0.01% NaN₃. Four-drop fractions were collected in scintillation

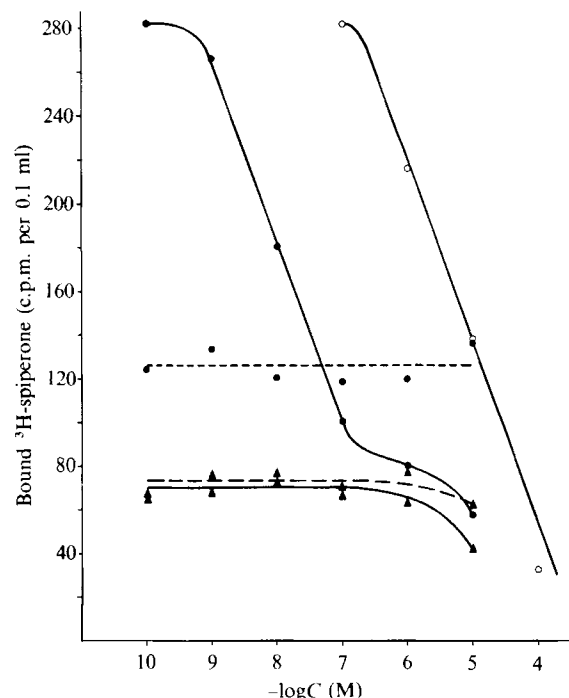


Fig. 1 Inhibitory effect of (+)butaclamol (●—●) and (-)butaclamol (○—○) on the binding of ³H-spiperone to solubilised material from dog striatum. Control experiments with (+)butaclamol used cerebellum extract (▲—▲) and thermally inactivated (56 °C for 10 min) preparations from dog striatum (●—●) and cerebellum (▲—▲). The values given are from one typical experiment.

vials and then counted for radioactivity. The gel-filtration procedure enabled the bound ³H-spiperone macromolecular complex to be separated from the free drug, as described in more detail previously^{12,14}.

Figure 1 shows that the ³H-spiperone binding in the solubilised preparation from dog striatum revealed a very pronounced stereospecific effect; (+)butaclamol competed with the binding at a concentration 300 times lower than (-)butaclamol, the inactive enantiomer. The inhibitory effect of

Table 1 Inhibition of ³H-spiperone binding in solubilised and membrane preparations from dog and rat striatum

	Dog striatum			Rat striatum		
	Soluble* A	Membrane B	IC ₅₀ (M) A/B	Soluble* C	Membrane† D	C/D
Spiperone	5.1 × 10 ⁻⁹	1.8 × 10 ⁻⁹	2.8	5.0 × 10 ⁻⁹	1.3 × 10 ⁻⁹	3.8
Benperidol	1.2 × 10 ⁻⁸	5.1 × 10 ⁻⁹	2.4	1.7 × 10 ⁻⁵	4.5 × 10 ⁻⁹	3,778
Lysuride	1.3 × 10 ⁻⁸	6.0 × 10 ⁻⁹	2.2		5.0 × 10 ⁻⁹	
(+)Butaclamol	2.0 × 10 ⁻⁸	1.5 × 10 ⁻⁸	1.3	6.2 × 10 ⁻⁶	2.0 × 10 ⁻⁸	310
Haloperidol	3.7 × 10 ⁻⁸	1.6 × 10 ⁻⁸	2.3	8.5 × 10 ⁻⁶	2.0 × 10 ⁻⁸	425
Fluspirilene	4.0 × 10 ⁻⁸	4.0 × 10 ⁻⁸	1.0	7.1 × 10 ⁻⁷	2.3 × 10 ⁻⁸	31
Flupenthixol	1.3 × 10 ⁻⁷	4.0 × 10 ⁻⁸	3.3	2.0 × 10 ⁻⁵	5.9 × 10 ⁻⁸	339
(±)2-(N,N-Dipropyl) amino-5,6-dihydroxytetralin	4.8 × 10 ⁻⁷	1.1 × 10 ⁻⁷	4.4		1.3 × 10 ⁻⁷	
Chlorpromazine	5.4 × 10 ⁻⁷	4.2 × 10 ⁻⁸	13		4.0 × 10 ⁻⁸	
Fentanyl	1.4 × 10 ⁻⁶	3.2 × 10 ⁻⁵	0.04	1.4 × 10 ⁻⁴	1.0 × 10 ⁻⁴	1.4
Sulpiride	1.6 × 10 ⁻⁶	5.6 × 10 ⁻⁷	2.9		8.7 × 10 ⁻⁷	
Pipamperone	2.9 × 10 ⁻⁶	1.3 × 10 ⁻⁶	2.2		1.3 × 10 ⁻⁶	
Dextimide	5.0 × 10 ⁻⁶	2.6 × 10 ⁻⁵	0.19	1.7 × 10 ⁻⁴	3.2 × 10 ⁻⁵	5.3
Mianserin	5.3 × 10 ⁻⁶	4.2 × 10 ⁻⁵	0.13		2.0 × 10 ⁻⁶	
(-)Butaclamol	5.5 × 10 ⁻⁶	1.3 × 10 ⁻⁵	0.42	3.4 × 10 ⁻⁵	2.1 × 10 ⁻⁶	16
R 5260	4.5 × 10 ⁻⁵	≥ 10 ⁻⁴		8.9 × 10 ⁻⁷	2.0 × 10 ⁻⁵	0.04
Propranolol	7.6 × 10 ⁻⁵	7.1 × 10 ⁻⁵	1.1	1.3 × 10 ⁻⁴	> 10 ⁻⁴	
Atropine	2.3 × 10 ⁻⁴	> 10 ⁻⁴			> 10 ⁻⁴	
Diazepam	2.5 × 10 ⁻⁴	1.3 × 10 ⁻⁴	1.9	> 10 ⁻⁴	> 10 ⁻⁴	

Inhibition was measured at five different concentrations and expressed as the IC₅₀ value (the drug concentration causing 50% inhibition of the stereospecific ³H-spiperone binding). The blank values were obtained using 2 × 10⁻⁶ M (+)butaclamol¹² except for the soluble preparation of rat striatum where the heat-inactivated preparation was used as blank¹².

*For spiperone, (+)butaclamol, haloperidol, flupenthixol, fentanyl and mianserin, the IC₅₀ values in solubilised dog striatum preparations are the mean values to two to five different experiments. This was also the case for the spiperone, benperidol, (+)butaclamol and (-)butaclamol IC₅₀ values in solubilised preparations from rats.

†Most of the values were taken from refs 4 and 6.

(+)-butaclamol was not observed when a cerebellum extract or a thermally inactivated preparation from striatum and cerebellum was used. The stereospecificity of the solubilised binding sites is quite comparable to that found in the membrane preparations from rat^{4,7-9} and dog striatum (Table 1). When compared with membrane fractions, the solubilised preparation from dog striatum was more rapidly inactivated by heat¹⁵. Table 1 shows that various dopamine antagonists belonging to different chemical classes (butyrophenone, phenothiazine, diphenylbutylamine, thioxanthene, procainamide and benzoquinolizine) and dopamine agonists (tetraline derivative⁶ and lysuride¹⁶) were nearly all of similar activity in soluble and membrane preparations from dog striatum (correlation coefficient $r = 0.94$), whereas anticholinergic, analgesic, a β -blocking agent, a minor tranquilliser and serotonin antagonists were practically inactive. Of the neuroleptic drugs, only chlorpromazine was relatively less active in the solubilised preparation, presumably because of its high affinity for nonspecific binding sites¹⁷⁻¹⁹.

In contrast to this, the IC_{50} values obtained using a solubilised preparation from rat striatum differed markedly from that obtained in membrane preparations. For example, benperidol displayed a 3,800-fold difference between the IC_{50} values of soluble and membrane preparations in rat striatum and only a twofold difference in the dog. Note that the affinities of dopamine antagonists and agonists are quite similar in membrane preparations of dog and rat. Interestingly, R 5260 (compound 25 of ref. 20), which is an analgesic (IC_{50} of fentanyl binding 1.0×10^{-9} M), was more active in inhibiting 3H -spiperone binding in the rat striatum soluble preparation than potent neuroleptic drugs like haloperidol and benperidol. Therefore, the binding sites solubilised from rat striatum displayed only a high affinity for spiperone or derivatives related to the spiro moiety (R 5260 and also compounds 2, 3, 4 of ref. 20). We assume that such binding sites correspond to the non-stereospecific displaceable sites detected in rat striatum using 3H -spiperone as ligand²¹.

As shown in Fig. 2, a good correlation between the spiperone binding in solubilised preparations and the antagonism of apomorphine-induced emesis was found for the dog striatum ($r = 0.89$) but not for the rat striatum. Spiperone was the only drug equiactive in solubilised preparations from both animals. Previous investigations clearly demonstrated that the haloperidol and spiperone binding of neuroleptic drugs correlated well with their pharmacological activities in the apomorphine test in rat⁶ and dog^{20,22,23} and their clinical potency¹⁷.

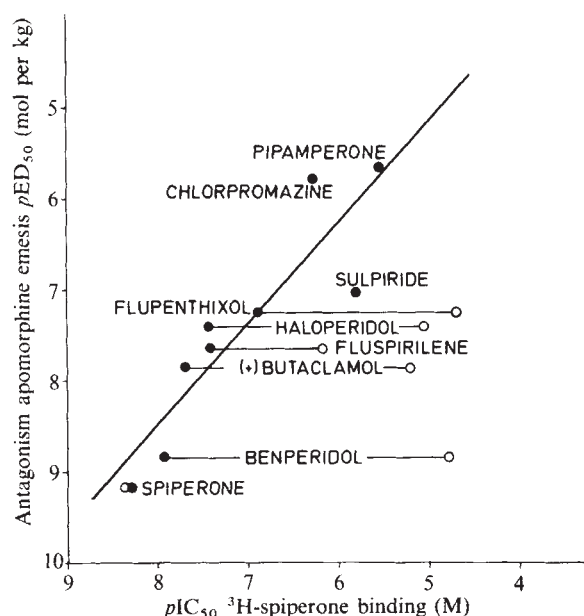


Fig. 2 Correlation between the IC_{50} values of 3H -spiperone binding in solubilised preparations from dog (●) and rat (○) and the antagonism of apomorphine-induced emesis in dog (see refs 22, 23).

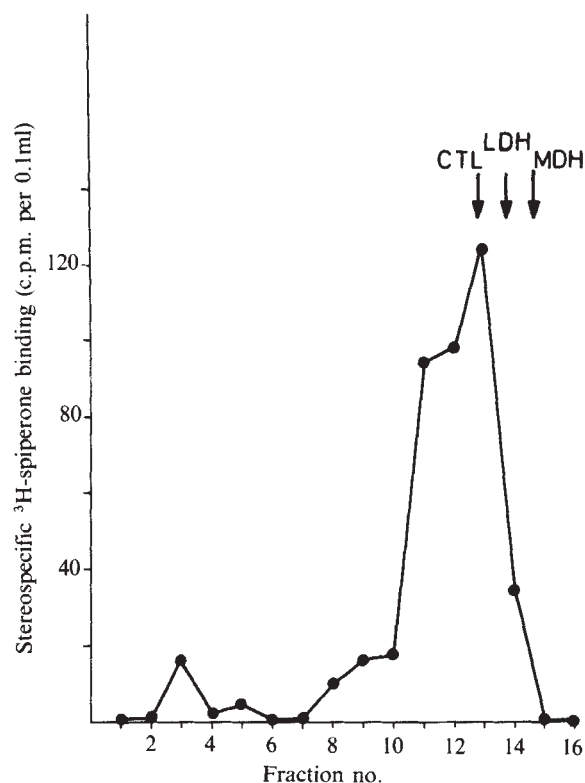


Fig. 3 Distribution of stereospecific 3H -spiperone binding after centrifugation of a digitonin extract from dog striatum. 1 ml of soluble extract was layered on 12 ml sucrose gradient (15–30%) buffered with 10 mM sodium phosphate (pH 7.2) containing 0.03% digitonin and 0.01% NaN_3 . Centrifugation was run at 2–3 °C in SW 40 Ti rotor (Spinco) at 30,000 r.p.m. (111,700g, r_{av}) for 16 h. Marker enzymes: catalase (CTL), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH).

Stereospecific 3H -spiperone binding in soluble dog striatum preparations was found to be saturable ($K_{D(app)}$ at 0 °C = 4.8 nM) and reversible ($t_{1/2}$ dissociation at 5 °C = 49 min). A more detailed kinetic analysis will be presented elsewhere¹⁵. To assess the macromolecular nature of these binding sites, the soluble extract from dog striatum was submitted to sedimentation centrifugation through a sucrose gradient and then compared with the sedimentation of marker enzymes. The peak of binding sites seemed to migrate more rapidly than malate dehydrogenase (4.3S) and lactate dehydrogenase (7.0S), but more closely resembled that of catalase (11.3S). When compared with the sedimentation properties of muscarinic receptor^{14,15}, the spiperone binding sites seemed to be more heterogeneous and to have a higher molecular weight (> 200,000).

The present results provide evidence that a macromolecular complex, able to bind 3H -spiperone stereospecifically, was solubilised from dog striatum. Most of the solubilisation criteria were fulfilled: not sedimentable at 262,000g (r_{av}) for 60 min, absence of lamellar membrane structure in electron microscopy²⁴, and low Svedberg coefficient compared with that of membranes.

Four lines of evidence indicate that the spiperone binding solubilised by digitonin from dog striatum and characterised by gel-filtration or sedimentation gradient is of dopaminergic nature. First, it was localised in the striatum but not in the cerebellum, and preliminary results showed that the binding sites solubilised from frontal cortex displayed a higher affinity for serotonin antagonists and a lower affinity for dopamine antagonists (in preparation). Second, in contrast to findings in the rat striatum, various dopamine antagonists from different chemical classes, chosen to display a wide range of activity, were nearly equiactive in the soluble and membrane preparation from dog striatum. Serotonin antagonists like pipamperone and mianserin were very poor inhibitors of 3H -spiperone binding to

soluble sites from striatum, whereas both drugs were very active in the frontal cortex⁶. Third, a tetraline derivative, known to be the most specific dopamine agonist^{6,25}, and thus being inactive in the spiperone binding assay in the frontal cortex, inhibited the spiperone binding of the solubilised preparation of dog striatum at very low concentrations. Finally, the good correlation between the inhibitory effects of drugs in the soluble preparation and their antagonism of apomorphine-induced emesis is only possible if the relative high affinity of the dopamine receptors is maintained after the solubilisation process.

Thus, the above results provide evidence that the spiperone binding sites solubilised by digitonin treatment from dog striatum retain the characteristics of dopamine receptors.

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Melanotropin potentiating factor is the C-terminal tetrapeptide of human β -lipotropin

WE recently demonstrated that the molar pigmentary potency of human β -lipotropin (β -LPH) is greater than other melanocyte-stimulating hormones (MSHs) on *Anolis* skin; it is 2.6-4.0 times greater than α -MSH¹ using the *Anolis* rate method of MSH bioassay². We also found (unpublished) human β -LPH to be 2 to 4 times more potent than α -MSH using the steady state (quantal³) method. This was due to a potentiation of the MSH sequence of β -LPH (LPH₄₇₋₅₃) by a factor associated with β -endorphin (LPH₆₁₋₉₁). We showed that the melanotropin potentiating factor (MPF) was not the opiate peptide, ⁵Met-enkephalin (LPH₆₁₋₆₅)⁴, and now report the identification of MPF as the sequence LPH₈₈₋₉₁.

Table 1 Intrinsic molar potencies of LPH peptide fragments calculated relative to α -MSH

LPH Structure (α -MSH)	Potency (1.000)
61-91	5.036×10^{-4} (4.813 and 5.270×10^{-4})
66-91	3.590×10^{-4} (3.406 and 3.785×10^{-4})
87-91	1.409×10^{-4} (1.377 and 1.442×10^{-4})
87(Bzl)-91	1.667×10^{-4} (1.574 and 1.765×10^{-4})
88-91	3.245×10^{-5} (3.067 and 3.434×10^{-5})
89-91	4.427×10^{-5} (4.101 and 4.780×10^{-5})
88-90	4.266×10^{-5} (4.027 and 4.520×10^{-5})
66-89	2.967×10^{-4} (2.797 and 3.148×10^{-4})
61-65, 70-89(D-Ala ⁶² , Leu ⁶⁵)	2.548×10^{-4} (2.432 and 2.670×10^{-4})
61-65, 76-89(D-Ala ⁶² , Leu ⁶⁵)	2.105×10^{-4} (1.992 and 2.225×10^{-4})
61-65, 81-89(D-Ala ⁶² , Leu ⁶⁵)	8.588×10^{-5} (8.269 and 8.920×10^{-5})
61-65, 85-89(D-Ala ⁶² , Leu ⁶⁵)	7.586×10^{-5} (7.205 and 8.199×10^{-5})
61-65, 88-89(D-Ala ⁶² , Leu ⁶⁵)	5.237×10^{-5} (4.921 and 5.573×10^{-5})
61-76	2.245×10^{-5} (2.095 and 2.406×10^{-5})
61-76 (D-Ala ⁶² , Leu ⁶⁵)	8.878×10^{-5} (8.375 and 9.412×10^{-5})
61-77	2.245×10^{-5} (2.095 and 2.406×10^{-5})
61-77 (D-Ala ⁶² , Leu ⁶⁵)	6.140×10^{-5} (5.884 and 6.408×10^{-5})
61-65	6.890×10^{-8} (6.764 and 7.017×10^{-8})

Dose-response curves were obtained to α -MSH and the 18 peptide sequences, using the *Anolis* rate method of MSH bioassay². Using analysis of variance⁸, the slope of the dose-response curves of the LPH peptide fragments did not differ significantly from that of α -MSH ($P > 0.05$). The 95% fiducial limits of the estimated potencies are given in parentheses.

The *Anolis* rate method of MSH bioassay² was used in these experiments. The peptides Tyr-Lys-Lys-Gly-Glu (human β -LPH₈₇₋₉₁), its (tyrosyl) *O*-benzyl derivative [87(Bzl)-91], Lys-Lys-Gly-Glu (89-91), Lys-Gly-Glu (88-91), and Lys-Lys-Gly (88-90) were prepared by classical solution methods and characterised by TLC, paper electrophoresis and amino acid analysis of acid and/or enzymatic digests. Other peptides used in

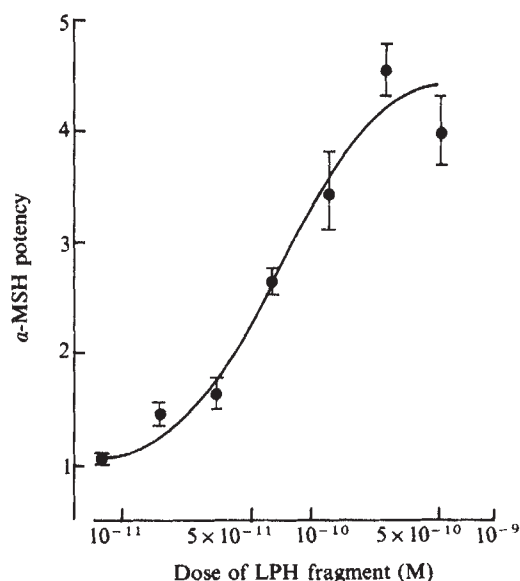


Fig. 1 The dose-related potentiation of α -MSH potency by β -endorphin. Dose-response curves were obtained from eight two-fold dilution series of α -MSH, seven of which had had added β -endorphin concentrations. The potencies of each α -MSH dose-response curve in the presence of β -endorphin concentration was calculated relative to that in the absence of added β -endorphin. Thus each point on the graph represents the increase in α -MSH potency with a β -endorphin concentration. The 95% fiducial limits of the estimated potencies are represented as vertical bars. There was a significant increase in α -MSH potency with concentrations of 16×10^{-12} M β -endorphin or greater ($P < 0.01$).

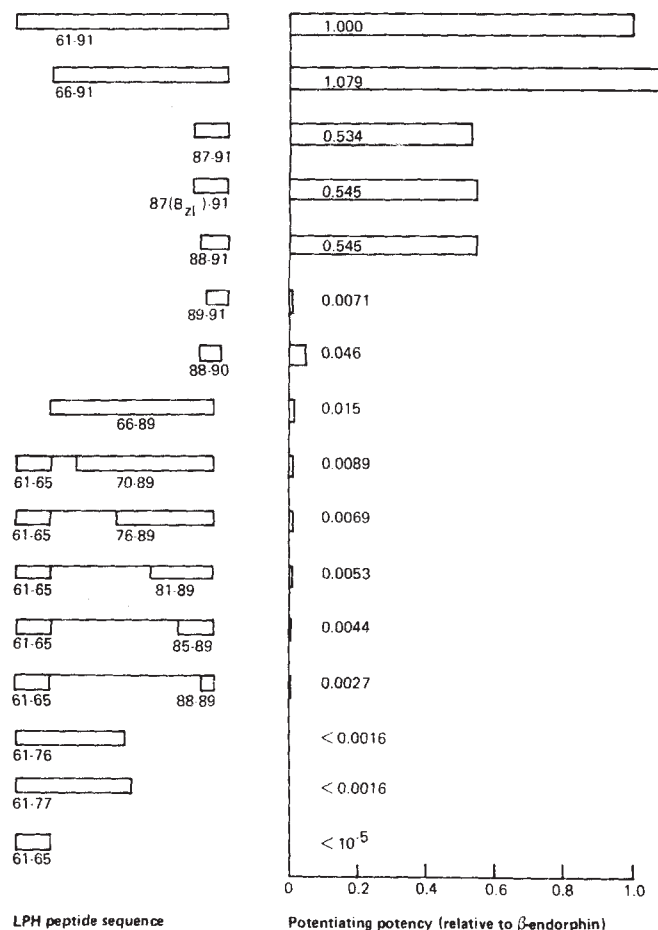


Fig. 2 Potentiating activities of LPH fragments on α -MSH potency. Bioassays of α -MSH were performed with and without LPH peptide fragments in various concentrations. The dose-related potentiation of α -MSH potency by each LPH fragment was measured as described in Fig. 1 and calculated relative to that of β -endorphin (LPH₆₁₋₉₁) using a two-factorial assay system.

these experiments were prepared by N. N. Petter (ICI) by solid phase methods⁵.

Dose-response curves were obtained for α -MSH and the synthetic LPH peptide fragments and their intrinsic molar potencies were calculated (Table 1). All showed negligible intrinsic MSH activity. Constant concentrations of the LPH peptide sequences were then incorporated into the twofold dilutions used to obtain the α -MSH dose-response and their relative potencies were calculated. β -Endorphin potentiated α -MSH potency with the dose-response curves remaining parallel. This potentiation was dose-related (Fig. 1) and the α -MSH potency was increased to a maximum of 4.5-fold. To determine the sequence responsible for this potentiation, the potentiation activity of each peptide sequence was compared with that of β -endorphin (Fig. 2). Unequivocally, the results show that the sequence responsible for the potentiation is LPH₈₈₋₉₁, Lys-Lys-Gly-Glu. Thus potentiating activity was abolished by the removal of the 88th or 91st amino acids from the tetrapeptide, while extension of the sequence to include tyrosine at position 87 did not increase potentiating activity further. The higher potentiating activity of LPH₆₁₋₉₁ (β -endorphin) and LPH₆₆₋₉₁ than LPH₈₈₋₉₁ was probably due to increased stability of the longer peptides as there was negligible activity in the sequences between LPH₆₁ and LPH₈₉. These findings cannot be attributed to characteristics of the rate assay as we have obtained similar results (unpublished) with a steady state (quantal³) method. We therefore conclude that the melanotropic potentiating factor (MPF) is human LPH₈₈₋₉₁, Lys-Lys-Gly-Glu. Whether MPF acts by stimulation of an independent receptor or by causing conformational changes is uncertain.

As a 16×10^{-12} M concentration of β -endorphin will potentiate MSH activity significantly (see legend to Fig. 1), the effect may be physiological. Although we have tested MPF activity on the *Anolis* skin, the unexpectedly high sebotropic⁶ and neurotropic⁷ potencies of β -LPH in the rat suggest that MPF may also modulate MSH peptide actions in the mammal and we are presently investigating this possibility.

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Inhibition of DNA synthesis *in vitro* by binding of benzo(a)pyrene metabolite diol-epoxide I to DNA

IT has been shown that benzo(a)pyrene (BP) is a strong mutagen and carcinogen after metabolic activation by mixed function oxidases and epoxide hydratase. Evidence now indicates that ($\pm$)-7,8-dihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol-epoxide I) is an ultimate carcinogenic and mutagenic form of BP¹⁻⁴. This metabolic intermediate interacts covalently with nucleic acids⁵⁻⁸. Most binding to DNA, 80% of the total, occurs by coupling between the 2-amino group of guanine and the carbon 10 position of diol-epoxide I (ref. 5). Binding to adenine, less than 15% of the total, leads to partial denaturation of the DNA double helix⁶, and binding of diol-epoxide I to the phosphate groups of DNA results in DNA strand scission⁸. Intercalation of diol-epoxide I may cause conformational change of the DNA double helix^{6,7}. Despite these extensive studies, the mechanism of alteration of the genetic function of DNA due to the binding of BP is not understood. We describe here a system which has enabled us to analyse the effect of BP binding to DNA on the replication of double-stranded circular DNA *in vitro*. pBR322 DNA (molecular weight, 2.6×10^6) is an artificial plasmid DNA derived from ColE1 and pBR313 in *Escherichia coli*⁹. It carries the base sequence derived from ColE1 DNA at the site of origin of replication⁹. Like ColE1 DNA¹¹⁻¹³, pBR322 DNA replicates semiconservatively and completely in a crude lysate of *E. coli*.

The binding of diol-epoxide I to pBR322 DNA was performed as described previously⁶. The number of diol-epoxide I molecules covalently bound to DNA, the molar ratio (MR), was determined using the radioactivity of ¹⁴C-di-epoxide I (29.4 mCi mmol⁻¹, NCI), UV absorption (254 nm), fluorescence and the molecular weight of pBR322 DNA. The covalent binding increased linearly as a function of increasing dose of diol-epoxide I concentration in the reaction mixture. High pressure liquid chromatography (Waters Bondapak C18

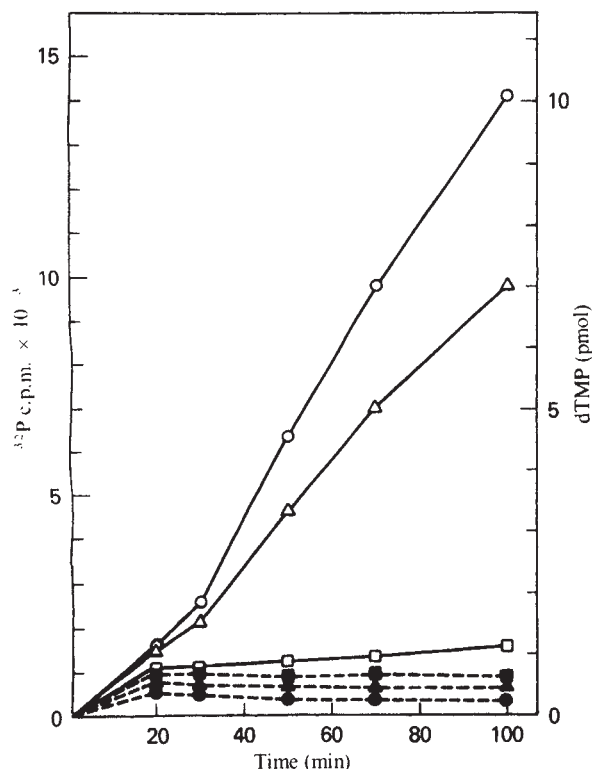


Fig. 1 Assay of the incorporation of ^{32}P -TMP into pBR322 DNA^{11,13}. *E. coli* YS1 cells were grown in 600 ml OC medium enriched by 0.4% Bacto-Peptone (Difco) and 0.1% yeast extract (Difco). When the number of cells reached 2×10^8 per ml, chloramphenicol was added ($180 \mu\text{g ml}^{-1}$). The culture was then incubated for 2 h. The cells were washed with 50 mM potassium phosphate buffer (pH 7.4), suspended in 0.6 ml of 15% sucrose-75 mM potassium phosphate buffer (pH 7.4) and frozen in a dry ice-isopropanol bath. The crude cell extract was prepared by thawing cells in an ice-water bath, and the volume adjusted to 1.5 ml with 10% sucrose-50 mM phosphate buffer (pH 7.4). To the cell suspension 60 μl 2 M KCl, 150 μl lysozyme (4 mg ml^{-1}) and 40 μl 5% Brij 58 were added at 0°C and mixed with a vortex mixer. After 30 min at 0°C, the mixture was centrifuged at 30,000 r.p.m. for 30 min in a Beckman SW 50.1 rotor. The standard assay mixtures (200 μl) were 25 mM potassium phosphate buffer, 67 mM KCl, 7.5 mM MgCl_2 , 0.2 mM each of rNTPs, 25 μM each of dNTPs, 2 mM spermidine, and contained 2 μg pBR322 DNA, ^{32}P -TTP (0.2 μCi) and 40% cell extract. The assay was performed at 30°C. 30 μl aliquots were taken at the times shown, precipitated with 10% trichloroacetic acid-0.1 M pyrophosphate, collected on filters (Whatman GF/C, 25 mm) and washed with 0.1 M HCl followed by ethanol. The open symbols indicate the standard assay mixture. Closed symbols indicate that rifampicin was added to the assay mixture. Control pBR322 DNA (MR = 0, $\circ$); pBR322 DNA-bound diol-epoxide I (MR = 1.4, $\triangle$); pBR322 DNA-bound diol-epoxide I (MR = 5.8, $\square$).

column, H_2O to 100% methanol gradient) of late elutant (~570 ml) from Sephadex LH-20 column chromatography of enzymatically hydrolysed DNA supported our calculation of the MR (data not shown).

Incubation of unmodified pBR322 DNA (control) in an extract from *E. coli* cells resulted in the incorporation of ^{32}P -TMP into the acid-precipitable fraction (Fig. 1). The incorporation increased linearly for at least 90 min. This incorporation was inhibited completely by the addition of rifampicin ($25 \mu\text{g ml}^{-1}$) at zero time. Rifampicin inhibits RNA-primed initiation of DNA synthesis but does not affect the elongation of the deoxypolynucleotide chain¹¹. When DNA covalently bound to diol-epoxide I (MR = 1.4) was used, the incorporation of ^{32}P -TMP was reduced about 30% (Fig. 1). From MR = 0 to MR = 5.9 the rate of reduction was linear with the number of diol-epoxide I molecules bound to one pBR322 DNA molecule.

When MR = 5.9 the initial incorporation (up to 20 min) was normal, but the total ^{32}P -TMP incorporation after 100 min was almost completely inhibited.

To study the mechanisms whereby DNA synthesis is inhibited by the binding of diol-epoxide I, we analysed the newly synthesised daughter strand DNA by sucrose density gradient centrifugation. The closed circular DNA synthesised *in vitro* appeared as a single peak with a skewed portion towards higher molecular weight in a neutral sucrose density gradient. Evidently the skewed portion represents closed circular DNA associated with newly synthesised DNA fragments. The amount of the closed circular DNA synthesised from DNA bound to diol-epoxide I (MR = 1.4) was about 30% less than that of the control (data not shown).

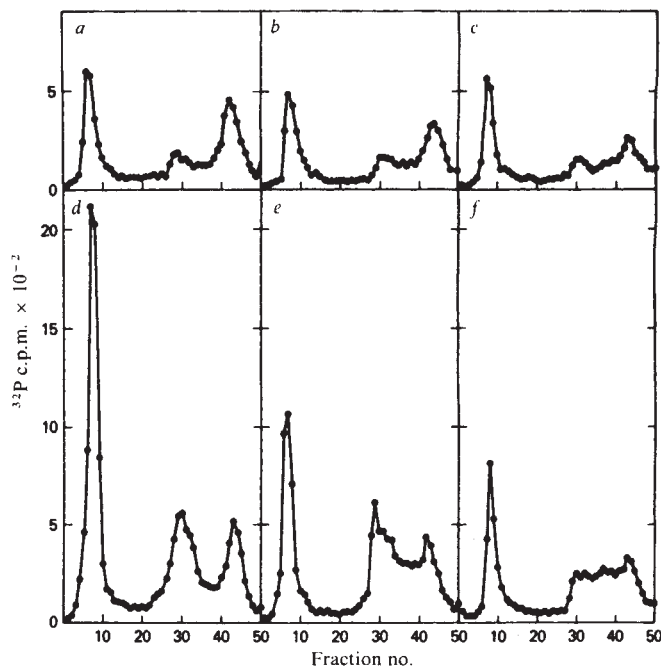


Fig. 2 Analysis of newly synthesised DNA products in alkaline sucrose density gradient. Assays were performed as described in the legend to Fig. 1. Samples were taken at 30 min (a-c) and 60 min (d-f). The reaction was stopped by the addition of one-fifth volume of 0.1 M EDTA (pH 8.0)-1% SDS. After addition of one-fifteenth of the original volume of 1.5 M NaCl-0.15 M sodium citrate, samples were treated twice with a chloroform-isomylalcohol mixture (24:1, v/v). After the centrifugation (5,000 r.p.m., 30 min at 4°C), the aqueous phase was dialysed against 50 mM Tris-HCl (pH 8.0)-50 mM EDTA-0.5 M NaCl. A 0.1 ml aliquot of each dialysed sample was treated with 0.24 M NaOH and applied to an alkaline sucrose density gradient (5-20%)¹⁶. a, d, Control, MR = 0; b, e, MR of diol-epoxide I is 1.4; c, f, MR of diol-epoxide I is 5.8.

Figure 2 shows the alkaline sucrose density gradient profile of the product DNA. In normal conditions (MR = 0), the newly synthesised closed circular DNA banded near the bottom of the gradient (fractions 6-8). Unit sized single linear and single circular molecules banded at fractions 30 (15S). A peak which appeared at the top of the gradient (fractions 42-47) represents the 6S initiation fragments dissociated from the origin of pBR322 DNA replication^{11,13}. The size classes of DNA synthesised during the first 30 min of incubation are identical and unrelated to the number of diol-epoxide I molecules bound to template DNA as observed. The only exception is that more 6S fragments accumulated in unmodified pBR322 DNA than in modified DNA, indicating that the formation of initial 6S fragments was slightly inhibited by diol-epoxide I binding.

After 60 min of incubation, about four times more closed circular molecules had been synthesised from unmodified DNA than after 30 min (Fig. 2a, d). When diol-epoxide I was bound to DNA at MR = 1.4, the closed circular DNA synthesised was only doubled after an additional 30 min incubation (Fig. 2b, e). When MR = 5.8, the amount of closed circular DNA did not increase after an additional 30 min incubation (Fig. 2c, f). The number of unit-sized single-stranded linear or circular molecules (15S) markedly increased when intact DNA was subjected to replication as shown in Fig. 2a, d. This class of DNA also increased as much as that in the control experiment from 30–60 min when the DNA was bound to 1.4 molecules of diol-epoxide I (Fig. 2b, e). However, the peak at fractions 26–32 (15S) in Fig. 2e is markedly skewed towards the top of the gradient. Moreover, when the molar ratio was 5.8, there was no distinctive peak observed in the region of fractions 26–33 (Fig. 2f). In contrast to other classes of DNA, the relative proportion of 6S molecules synthesised did not change significantly in either control or modified DNA during an additional 30 min incubation. These results indicate that many intermediate sized newly synthesised DNA fragments with length less than the unit size of pBR322 DNA were accumulated when diol-epoxide I modified DNA was subjected to replication. The effect of covalently-bound diol-epoxide I is therefore to inhibit chain elongation with little effect on the initiation of DNA replication.

Note that pBR322 DNA, which carries the same base sequence as ColE1 DNA at the initiation site, consists of a high proportion of adenine and thymine residues¹⁰, which have significantly less chemical reactivity with diol-epoxide I than guanine residues^{5–7}. Based on these findings and other studies, it may be reasonable to assume that the initiation site of DNA replication is free from the binding of diol-epoxide I and is therefore capable of generating a normal initiation of DNA synthesis when the molar ratio is small. The elongation of the deoxypolynucleotide chain was effectively blocked at the binding site of the template molecule. Hsu *et al.* have reported similar results regarding the inhibition of converting single-stranded Φ X174 DNA to double-stranded circular DNA¹⁴.

Another question is whether the binding of diol-epoxide I completely blocks the elongation of the nascent DNA chain or whether the binding site can be excised and repaired, allowing

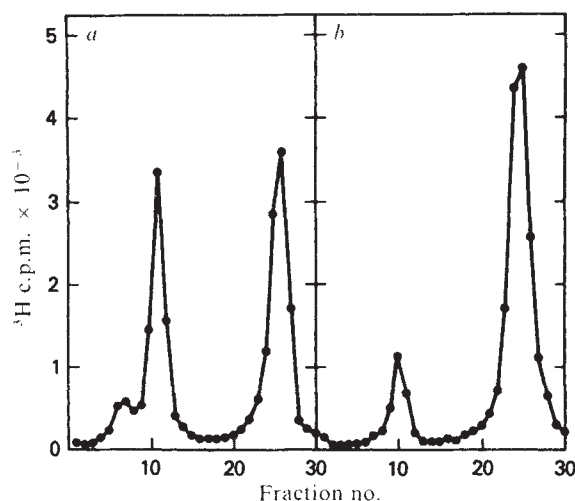


Fig. 3 Analysis of heat-treated ColE1 DNA modified with diol-epoxide I in an alkaline sucrose density gradient centrifugation. Tritium-labelled ColE1 DNA was prepared after amplification with chloramphenicol as described previously¹⁶. The purified ColE1 DNA was modified with diol-epoxide I as described in the text. Unmodified ColE1 DNA (a) and modified ColE1 DNA (MR = 1.4) (b) were treated at 90 °C for 5 min in 5 mM Tris-HCl (pH 8.0)–5 mM EDTA–50 mM NaCl, and then the pH was adjusted to pH 12 with 4 M NaOH. Samples were analysed by the 5–20% alkaline sucrose density gradient as shown in the legend to Fig. 2.

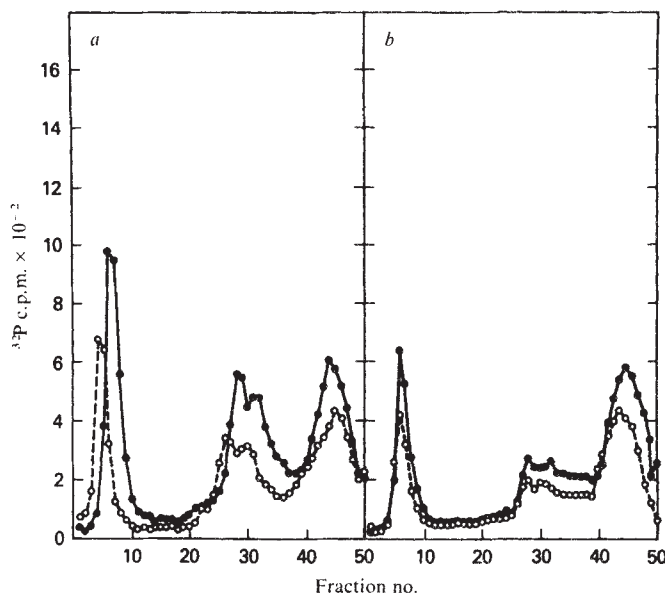


Fig. 4 Newly synthesised pBR322 DNA with (a) and without (b) diol-epoxide I (MR = 0 and 1.4 respectively) was analysed in an alkaline sucrose density gradient before and after heat-treatment. pBR322 DNA was subjected to the *in vitro* assay system for 60 min as described in the legend to Fig. 1. The product was analysed in the alkaline sucrose density gradient centrifugation with (dashed line) and without (solid line) heat-treatment as shown in the legend to Fig. 3.

the completion of replication. To investigate this, a method was devised to detect a small number of binding sites on closed circular DNA. About 50% of the closed circular DNA (MR = 0) was converted to single-stranded circular and linear forms in an alkaline sucrose density gradient after heat treatment of the DNA at 90 °C for 5 min (Fig. 3a). The conversion of 50% is a result of alkaline hydrolysis of the RNA segment which is presented in chloramphenicol-amplified ColE1 DNA¹⁵. The rate of conversion was clearly increased when diol-epoxide I was bound to ColE1 DNA (MR = 1.4), heated and analysed in an alkaline sucrose density gradient (Fig. 3b), indicating that the binding site itself is heat-alkali-labile. This provides a sensitive assay method for the detection of bound diol-epoxide I on DNA strands. When the method was applied to the DNA which had completed a round of replication (for method see legend to Fig. 2), the rate of conversion from closed circular to the other forms was no different for the control and modified (MR = 1.4) DNA (Fig. 4).

These results suggest that the parent strands no longer possess diol-epoxide binding sites which were present before replication and also rule out the possibility of the binding sites in the template being bypassed during replication. Excision of diol-epoxide-damaged DNA from the parental strand would best explain the reduction in the rate of DNA elongation described earlier.

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Suppression of a yeast *amber* mutation in *Escherichia coli*

THE complementation of *Escherichia coli* auxotrophs by cloned eukaryotic genes¹⁻⁴ makes it possible to use standard bacterial genetic techniques to study these eukaryotic genes. Here, we describe the cloning of a *Saccharomyces cerevisiae* (yeast) gene with an *amber* suppressible allele. Reversion and suppression properties of this allele are examined in growing *E. coli* cells.

The yeast *his3* gene coding for imidazoleglycerolphosphate (IGP) dehydratase was cloned as a hybrid of bacteriophage λ which complemented *hisB* auxotrophs of *E. coli* lacking the analogous activity¹. The wild type yeast *his3* gene is transcribed and translated in *E. coli* with high fidelity to produce an enzyme activity strongly resembling the activity found in yeast cells². Complementation analysis of many derivatives containing *his3* sequences indicates that the *his3* structural gene is localised to a region of approximately 700 base pairs⁵. The length and location of this *E. coli* complementation unit is similar to the region homologous to the 650 base poly(A)-containing RNA species found in yeast cells (K. S., unpublished).

Genetic analysis in *E. coli* of the yeast *his3* gene depends on the availability of cloned derivatives which are nonfunctional. Mutant *his3* genes which are nonfunctional in *his3*⁻ yeast cells are also nonfunctional when cloned and propagated in *E. coli*; that is, they do not complement *E. coli hisB* auxotrophs². The order of two such *his3* lesions has been established by a three-factor cross of the bacteriophage λ hybrids containing the cloned mutant genes². Non-complementing deletions of the yeast *his3* gene spontaneously generated during lytic growth of a bacteriophage $\lambda his3$ hybrid have been isolated⁵ by the method of Parkinson and Huskey⁶. The physical locations of the yeast lesions have been determined by deletion mapping in *E. coli*⁵. Deletion mutants which require transcriptional initiation from the λ promoter P_L for *his3* expression have been used to define a yeast DNA sequence which functions in *E. coli* as a promoter⁷. Derivatives of a $\lambda his3$ hybrid which overproduces IGP dehydratase activity in *E. coli* have also been isolated (M. Brennan and K. S., unpublished results).

The demonstration that cloned yeast genes can be reintroduced into yeast cells by transformation and expressed⁸

makes it possible to fuse the genetic systems of *E. coli* and *S. cerevisiae*. Recent findings indicate that DNA transformation of yeast can occur by at least three mechanisms (ref. 9 and J. B. Hick, A. Hinnen and G. R. F., unpublished results). Depending on the particular mechanism, transforming DNA can integrate into the yeast chromosomes by homologous recombination and/or replicate autonomously. Introduction of physically and genetically defined cloned *his3* derivatives into yeast cells should be an important tool in elucidating mechanisms of yeast regulation, DNA replication and recombination.

To aid such studies it would be useful to clone a conditionally lethal yeast mutation. Strain constructions would be significantly facilitated and interpretation of experimental results would be strengthened by the conditional nature of the lesion. To isolate a conditionally lethal lesion, 112 independently derived *his3* mutants of yeast¹⁰ were screened for *amber* suppressibility by scoring for histidine prototrophy following mating with *his3*-532 SUP4-2. Three mutants (*his3*-14, *his3*-19 and *his3*-X5-21B) presumably containing internal UAG codons suppressible by tyrosine-inserting tRNAs¹¹ were isolated. DNA from strain *his3*-X5-21B was cloned in λ gt4 (ref. 12) by the *Eco*RI-DNA ligase method². From this pool of λ gt4 hybrids, a phage containing *his3* sequences was isolated by the plaque filter hybridisation method of Benton and Davis¹³. The 10.1 kb (kilobase pair) *Eco*RI fragment in this phage, Sc2693, is physically indistinguishable from the analogous fragment (Sc2601) which complements *E. coli hisB* mutations. λ gt4-Sc2693 does not complement *hisB*463; therefore, the phage contains a cloned yeast *his3* gene with an *amber* lesion. The *his3* gene is located internally within a 1.7 kb *Bam*HI fragment of Sc2601 DNA⁵. The equivalent *Bam*HI DNA fragments of λ hybrids containing *his3* mutant genes were cloned in the yeast vectors YIp5 and YRp7 (ref. 9). The sources of these cloned mutant genes were λ gt1-Sc2612 (*his3*-38), λ gt6-Sc2679 (*his3*-532) (ref. 2), and λ gt4-Sc2693 (*his3*-X5-21B).

All mutant *his3* genes cloned in these yeast vectors were introduced into the *E. coli* strain *hisB*463 by selection for the plasmid-coded gene for ampicillin resistance. The spontaneous reversion rates in *E. coli* of these yeast lesions were measured and compared to the spontaneous reversion rates in yeast (Table 1). The comparison is complicated by the fact that in these *E. coli* cells, the *his3* genes are presumably present in about 20 copies, and that the expression is only about four times above the single copy level⁷. The reversion rates are measured as His⁺ colonies obtained per cell. It is unclear how these numbers are related to number of reversion events per DNA molecule. Multiple copies of *his3*-38 in *hisB*463 cells allow some growth in the absence of histidine. The reversion rate of this lesion in *E. coli* as determined from plasmid hybrids (2×10^{-8}) agrees well with the previous results obtained with phage hybrids². The reversion rate of this lesion in yeast is significantly lower ($<10^{-9}$). *his3*-532 is a very stable lesion in both organisms, though revertants are detected in *E. coli* but not in yeast *his3*-X5-21B reverts in *E. coli* at a significantly lower rate than it reverts in yeast. These results indicate that spontaneous reversion of a given yeast lesion occurs at different rates in yeast and in *E. coli*.

Table 1 Suppression and reversion characteristics of yeast *his3* lesions

<i>his3</i> lesion	λ Hybrid	Plasmid hybrid	<i>hisB</i> (<i>supF</i>)	<i>his</i> ⁺ colonies <i>hisB</i>	Yeast
<i>his3</i> -38	λ gt1-Sc2612	YIp5-Sc2719	2×10^{-8}	3×10^{-8}	$<10^{-9}$
		YRp7-Sc2719	1×10^{-8}	1×10^{-8}	
<i>his3</i> -532	λ gt6-Sc2679	YIp5-Sc2720	2×10^{-10}	1×10^{-10}	$<10^{-10}$
		YIp5-Sc2721	1	3×10^{-9}	
<i>his3</i> -X5-21B	λ gt4-Sc2693	YRp7-Sc2721	1	1×10^{-9}	2×10^{-7}

As *his3-X5-21B* is suppressed in yeast by a tyrosine-inserting tRNA, suppression in *E. coli* was examined with an analogous tRNA species (*supF*). *hisB463* cells containing the cloned yeast *his3* lesions were lysogenised by a phage ($\phi 80$ *supF*) containing the *E. coli* gene coding for a tRNA species capable of suppressing amber (UAG) codons¹⁴. Introduction of the *supF* allele into these *hisB463* derivatives was determined by their ability to plate λ C1857Sam7. *hisB463* cells containing both the *his3-X5-21B* and the *supF* alleles grow in the absence of histidine (Table 1); that is, the *his3* lesion is suppressed by *supF*. This result is not due to an artefact of selection, as both the *his3-X5-21B* and the *supF* alleles were introduced into the *hisB463* host by non-selective means. Furthermore, it confirms the amber suppressibility of the *his3-X5-21B* allele. It is now possible to introduce DNA containing this conditionally lethal yeast gene back into yeast and *E. coli*.

These results indicate that an *E. coli* amber-suppressing tRNA species suppresses a yeast amber lesion in growing *E. coli* cells. It has been previously established that purified yeast suppressor tRNAs, when present in heterologous, eukaryotic, *in vitro* translation systems, suppress prokaryotic (Q β) amber mutations^{15,16}. Taken together, these *in vivo* and *in vitro* experiments provide evidence that translational termination and tRNA suppression of nonsense codons are processes which are conserved between prokaryotic and eukaryotic organisms.

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Genotype control of the dystrophin gene in mice

MUTANTS of the dystrophin gene (*dy*) in mouse suffer from progressive skeletal muscle loss, together with inadequate repair or regeneration⁶. In 1974 I reported a distinctive phenotypic difference between two dystrophic mutant alleles with respect to myogenesis *in vitro*¹. Homozygous *dy*²³ mice gave cultures in which there was apparently normal myogenesis, whereas mice homozygous for *dy* gave cultures which contained large numbers of grouped uninucleate myoblasts

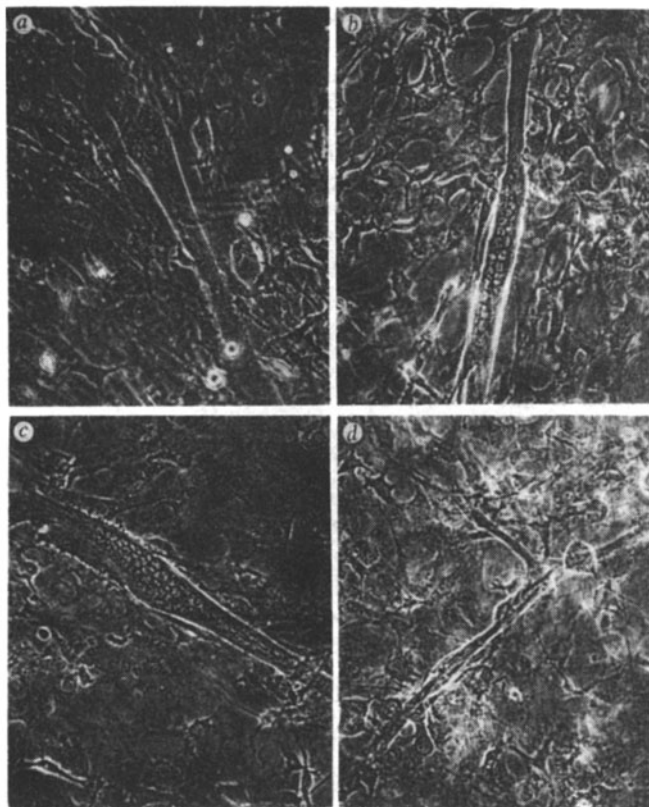


Fig. 1 Unstained living cultures photographed with phase contrast-optics. The structures are typical and the illustrations representative of the experimental results, *a*, C57 +/+, normal multinucleate myotube; *b*, C57 *dy*²³/*dy*²³, large syncytial myotube; *c*, C57 *dy*/*dy*, a definite multinucleate myotube; *d*, 129 *dy*/*dy*, characteristic pseudostraps with discrete uninucleate cells. All photographs were taken at 4 d *in vitro* in identical conditions.

(pseudostraps) in place of multinucleate syncytial myotubes². MacPike, however, showed³ that muscular dystrophies caused by these two alleles carried in the same genetic background (C57BL/6J) were similar when assessed histologically. I have, therefore, investigated how the process of myogenesis *in vitro* (which can be considered to be another independent function of the muscle) is affected not only by the two allelic mutants but also by the genetic background. The *dy* dystrophy was known to be histologically similar in either the C57BL/6J or 129/ReJ background³ but surprisingly C57BL/6J *dy*/*dy* cultures gave normal myogenesis while 129/ReJ *dy*/*dy* cultures again produced pseudostraps.

The mice used in this series of experiments were C57BL/6J *dy*²³/*dy*²³, C57BL/6J *dy*/*dy*, C57BL/6J +/+ and 129/ReJ *dy*/*dy* at 2, 3, 4 and 5 months of age. Cultures were obtained from 4 day crush lesions as described previously¹. Daily examination for a period of 8 days was carried out blind; 48 cultures of each genotype were assessed. As before, the pattern of growth during the first 3 days was one of proliferation and explant spreading. Myotubes, increasing in size and number, developed by days 4 to 8 in all three C57BL/6J genotypes including the *dy* mutant (Fig. 1*a, b, c*). The 129/ReJ *dy*/*dy* showed without exception the characteristic pseudostraps (Fig. 1*d*) and the occasional small myotube during the first 3 days.

In vitro the 129/ReJ *dy* muscle attempts to form myotubes but does not succeed¹. However, the same muscle in a more complex organotypic culture of fetal spinal cord and muscle exhibits some regeneration⁴ although it is much less than normal muscle in the same conditions. Furthermore, the *dy* muscle cells cultured as a low density monolayer⁵ produce apparently normal myogenic colonies which contain well differentiated muscle fibres. Clearly, myogenesis from dystrophic muscle is extremely susceptible to environmental conditions. But it is also

clear from the experiments reported here that there is a positive indication of a genotypic control over dystrophic myogenesis.

The consequence of these findings of the interaction between the gene at the *dy* locus and any other genes of a particular background is clearly important with regard to the interpretation of experiments, past and future.

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Sensitivity of low molecular weight RNA synthesis to UV radiation

RNA SPECIES C and D (nomenclature of Weinberg and Penman¹) are among the most abundant of the long-lived², homodisperse, low molecular weight nuclear RNAs³, and are present in all vertebrates tested⁴, but their function is unknown. They have several interesting characteristics: they seem to be transcribed from multiple-copy genes (10^2 – 10^3 genes per genome)^{5,6}, to pass through the cytoplasm for a few minutes shortly after their transcription^{3,7–10}, to have 5'-end caps that are very similar to those of eukaryotic mRNAs^{11,12}, and to be present in heterogeneous nuclear RNA-protein particles^{13,14}. Here, we show that C and D RNA syntheses are unexpectedly sensitive to UV radiation in HeLa cells. The effect of UV radiation on RNA synthesis has been used to study the organisation of transcription units¹⁵. UV irradiation, causing random formation of pyrimidine dimers in DNA, results in premature termination of transcription. The probability of causing a UV lesion within a transcription unit is directly proportional to its length, making it possible to measure the distance between a gene and its promoter. The present UV sensitivity data are compatible with the transcription units for the low molecular weight (less than 200-nucleotide long^{11,12}) RNA species C and D being as long as 5 kilobases.

In this study, RNA was extracted from whole cells and analysed by polyacrylamide gel electrophoresis. Figure 1 shows an example of the routine assay used. Recovery variations were corrected by the ¹⁴C-labelled RNA bands, as the cells had been incubated with ¹⁴C-uridine for 1 d before the experiment. The briefly labelled RNA migrating between 5S and 4S RNA seems to be mainly 4S RNA precursors^{16,17}, and so it was included in the estimations of newly made 4S RNA. If any RNA fragments were generated by UV effects on large transcription units¹⁸, they might have raised the radioactivity background in the gel electrophoresis pattern. This would not have affected our estimates, because only the counts of a given peak above this background were computed. These results would not have been affected by any possible effect of UV radiation on the cytoplasmic-nuclear maturation of C and D RNA, as whole cell RNA was assayed.

Figure 2a shows the sensitivity of the synthesis of 45S rRNA to UV radiation. It is assumed that brief labelling of RNA (20 min with ³H-uridine) approximates RNA synthesis, as precursor pools do not seem to be affected by UV radiation¹⁸. The UV dose that resulted in a 37% level of residual synthesis (D_{37}) was near 8 s when the incident dose rate was $34 \text{ erg mm}^{-2} \text{ s}^{-1}$.

The resulting product is almost identical to the D_{37} incident dose of 270 erg mm^{-2} reported for mouse L cell 45S rRNA synthesis¹⁹. Figure 2b and c shows that the synthesis of C and D RNA was inhibited, whereas the synthesis of 4S and 5S RNA was essentially unaffected at this dose range of UV radiation, as had been shown by Goldberg *et al.*²⁰. In addition, the inactivation curve for both C and D RNA synthesis deviated from exponential kinetics (Fig. 2b, c). The D_{37} value for both C and D RNA synthesis, calculated by first order approximation of the initial slope (broken straight lines in Fig. 2b, c), was nearly 30 s. At an incident dose rate of $34 \text{ erg mm}^{-2} \text{ s}^{-1}$, the D_{37} incident dose for C and D RNA synthesis is then about $1 \times 10^3 \text{ erg mm}^{-2}$. The D_{37} incident dose for 18S rRNA synthesis is 880 erg mm^{-2} (ref. 19), whereas the distal end of the 18S rRNA gene is located about 5 kilobases from its promoter, as determined by electron microscopic²¹ and UV transcription mapping¹⁹.

Two models could account for our results. Based on the D_{37} value, the C (or D) RNA sequences would be derived from 4–5-kilobase-long transcription units, and according to the deviation from exponential kinetics, several C (or D) RNA genes would be tandemly arranged behind a common promoter¹⁹. It can then be estimated that at least seven gene copies would exist per transcription unit¹⁹. Alternatively, the present data are compatible with the synthesis of a population of original transcripts which are heterogeneous in size, vary in length between approximately 0.5 and 5 kilobases, and which have a C (or D) RNA sequence at or near the 3'-terminus of each polynucleotide. Either model is unexpected because the known precursors to C and D RNA are about 0.2 kilobases long^{9,22}. The precursors of C and D RNA, which are only slightly larger than the mature species, can be detected after at least 4 min of labelling⁷. It therefore follows that the proposed large original transcripts would be cleaved shortly after or during transcription.

There is no detectable repair of the lesions responsible for premature termination of transcription of rRNA¹⁹, histone²³ and adenovirus type 2 (ref. 24) genes, for at least 60–90 min after UV irradiation of mammalian cells. Therefore, it seems unlikely that repair of UV damage would significantly affect our results in the 30 min after UV irradiation in the present experiments. In any case, the unusual feature of the present data is the high sensitivity to UV radiation of C and D RNA synthesis, and fast repair would have only masked some of this sensitivity. It could be argued that the proposed interpretations of the data might be erroneous because UV radiation may affect transcription by various eukaryotic RNA polymerases differently. It has already been shown that UV transcription mapping works well with transcription by RNA polymerases class I and II (refs 19, 20, 25, 26), and one of these two types of polymerase is suspected to be involved in the synthesis of C and D RNA^{27,28}. It

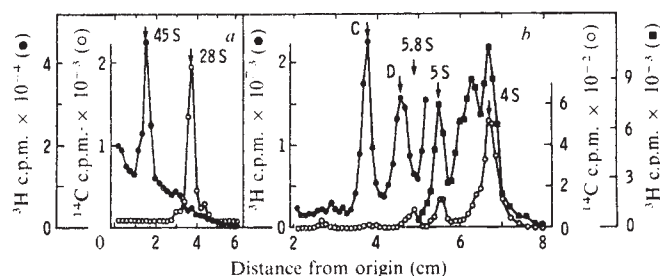


Fig. 1 Polyacrylamide gel electrophoresis patterns of large (a) and small (b) RNAs from whole cells. HeLa S3 cells were labelled for 1 d with ¹⁴C-uridine⁷, and then exposed to UV irradiation², placed at 15 cm from a GE G2578 lamp (incident dose rate of $34 \text{ erg mm}^{-2} \text{ s}^{-1}$). The cells were then spun down, and resuspended at $2 \times 10^6 \text{ cells ml}^{-1}$ in medium and serum; after 10 min at 37°C , [³H-5]uridine ($20 \mu\text{Ci ml}^{-1}$, 26 Ci mmol^{-1}) was added, and the cells were finally collected after labelling for 20 min at 37°C . RNA was extracted from whole cells with phenol and SDS at 55°C , and analysed by 2.4% (a) and 10% (b) polyacrylamide gel electrophoresis as described elsewhere²⁹. ■, ³H (expanded scale); ○, ¹⁴C.

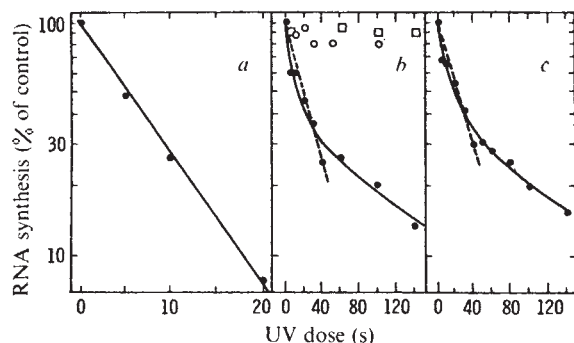


Fig. 2 Effect of UV radiation on labelling of several RNAs in HeLa cells. *a*, 4S rRNA; *b*, C RNA (●), 4S RNA (□) and 5S RNA (○); *c*, D RNA. The broken lines represent pseudo-first-order approximations to the experimental data. All conditions were as indicated in the legend to Fig. 1.

seems unlikely that C and D RNA could be synthesised by RNA polymerase class III, because in the presence of α -amanitin, when 5S and 4S RNA synthesis is essentially unaffected, the synthesis of C and D RNA is markedly inhibited²⁸.

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***Azotobacter* cytochrome *b*_{557.5} is a bacterioferritin**

FERRITIN is the presumed iron storage protein of mammalian¹, plant² and certain fungal³ systems. The nitrogen-fixing bacterium *Azotobacter vinelandii*, produces a *b*-type cytochrome containing large amounts of non-haem iron and no labile sulphide, as first shown by Bulen *et al.*⁴. Here we present

evidence which reveals this protein to be a ferritin-like species, thus constituting the first authenticated occurrence of ferritin in a bacterium. The interesting redox properties of this molecule, bacterioferritin-cytochrome, are presented and discussed.

The cytochrome *b*_{557.5} was prepared by the method of Bulen *et al.*⁴ from *N*₂-fixing *A. vinelandii* cells collected after they had entered the stationary phase. The purification method for this acidic protein involves heat treatment, protamine sulphate precipitation, dissolution by cellulose phosphate treatment and repeated crystallisation by the addition of Mg²⁺. The preparation is homogeneous on polyacrylamide gel electrophoresis, and SDS gel electrophoresis reveals a single subunit. The Fe content of 2.8–3.5 μ mol Fe per mg protein requires about 100 iron atoms per haem molecule in the preparation. As the iron constitutes 13–20% of the weight of the protein, we investigated the possible relationship of this protein to ferritin.

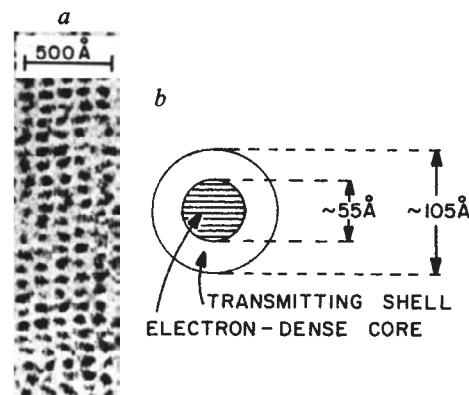


Fig. 1 *a*, Small portion of a transmission electron micrograph of a crystalline sample of bacterioferritin-cytochrome of *A. vinelandii*. Sample preparation involved fixation with glutaraldehyde, dehydration with ethanol and embedding in Spurr's low viscosity embedding medium. No stain was used. *b*, Schematic idealisation of the dense inner region and 'transparent' outer shell of the bacterioferritin-cytochrome as revealed in the electron microscope. The regions are assigned, respectively, to the Fe core and protein shell.

Table 1 compares various analytical properties of this molecule with those of horse spleen ferritin^{5–8}. SDS gel electrophoresis reveals a single subunit of molecular weight (MW) 17,000, slightly smaller than that of mammalian ferritin⁶. Amino acid analysis shows the expected dominance of acidic amino acids and yields a nearest integer MW of 17,185. Although the similarity to ferritin is already apparent, the most striking indication of the ferritin nature of the protein comes from electron microscopy of the unstained crystalline preparation. The portion of an electron micrograph shown in Fig. 1 reveals the presence of electron-dense cores of ~55 Å diameter within approximately spherical electron transmitting shells of diameter ~105 Å. The outer shells are presumed to be of protein, and the inner core must contain an iron compound (see below). These dimensions are virtually identical to those found by electron microscopy for mammalian ferritin⁹. Similarly, the apparent variable occupancy of the inner cores parallels that found in ferritin^{1–3,9}.

Crystalline samples of the molecule have been studied by Mössbauer spectroscopy of ⁵⁷Fe at natural abundance by Lang and Spertalian (in preparation), and reveal a quadrupole-split doublet characteristic of Fe³⁺ at 77 K. As the temperature is lowered to 1.5 K, a six-line magnetically split multiplet appears. The limiting high and low temperature spectra are virtually identical to those found previously^{10–12} for horse ferritin. Magnetic susceptibility of the *Azotobacter* protein at 34 °C by the Evans NMR method¹³ gives a magnetic moment, $\mu_{\text{eff}} = 3.7$ Bohr magnetons per Fe atom. The same experiment for horse spleen ferritin (Sigma) also yields 3.7 BM per Fe atom, in

agreement with previous work^{14,15}. The Mössbauer and susceptibility experiments together establish the weakly coupled high-spin ferric nature of the bulk iron.

The combined information from the mode of preparation and crystallisation, the high Fe content, the electron microscopy, the amino acid composition, the subunit MW, the Mössbauer spectra and magnetic susceptibility, lead us to conclude that this protein represents the first example of a bacterioferritin.

It was the presence of haem that led to the original purification of the protein⁴. The electronic spectrum of the oxidised form has a Soret band at 417 nm as the only distinct peak in the visible region⁴. On reduction with $\text{S}_2\text{O}_4^{2-}$ /methyl viologen, we find a clean reduced cytochrome spectrum with α , β and Soret bands at 557.5, 527 and 425 nm, respectively.

To show the intimate association of haem with this bacterioferritin, we note first that extensive dialysis and repeated recrystallisation can be accomplished without change in composition. Further, polyacrylamide gel electrophoresis, isoelectric focusing and gel-filtration chromatography each reveal a homogeneous preparation and establish the inseparability of haem and non-haem iron components of the protein. Although the haem has been identified as protoporphyrin IX (ref. 4), quantitative determination by the pyridine haemochromogen method is precluded by variable absorption (or occlusion) of haem by the precipitated non-haem iron cores. Haem was therefore determined by treatment with formic acid, which unfolds the protein, solubilises the non-haem iron and presumably stabilises the ferrihaem as the formic acid complex. With protein determined by the Lowry procedure¹⁶ and haem absorption in formic acid standardised with haemin chloride or haemoglobin, we initially find an average of 37,000 daltons per haem. We note that one haem for two subunits requires 34,000 daltons per haem. In addition to the apparent inseparability of the haem and non-haem iron and the roughly constant haem/protein ratio, the redox properties of the protein show an intimate relationship of haem and non-haem iron.

Coulometric titrations¹⁷ (Fig. 2) reveal a reversible Nernst plot (for $n = 1$) with a potential of -416 mV at pH 7 using methyl viologen as the mediator. Coulometry shows that for most samples one electron is taken up for each iron atom present in the molecule. Spectroscopy reveals that both the haem and the large excess of non-haem iron are reduced in this process. Potentiometric titration of the protein, carried out by monitoring the appearance of the α band of the reduced haem, reveals that the haem undergoes reduction at a potential only 30 mV more negative than that of the bulk iron. Thus, both the haem and non-haem iron are reducible at similar extremely low redox potentials. These potentials are significantly more negative than those required to reduce horse spleen ferritin.

We have preliminarily explored the fate of the reduced *Azotobacter* protein. Our initial experiments show that the coulometrically fully reduced protein can be passed through an

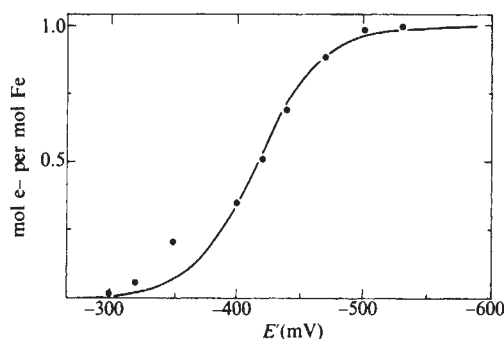


Fig. 2 Coulometric titration of bacterioferritin-cytochrome of *A. vinelandii* at 25 °C and pH 7 versus the normal hydrogen electrode using methyl viologen as mediator by the method described in ref. 12. The points are experimental values and the curve is calculated from the Nernst equation for $n = 1$, $E' = -416$ mV.

Table 1 Comparison of *Azotobacter* protein with horse spleen ferritin

Amino acid	<i>Azotobacter</i> bacterioferritin-cytochrome	Horse spleen ferritin
Cys	2.3	2.9
Asx	17.2	17.3
Thr	2.2	5.5
Ser	5.3	9.0
Glx	26.8	23.9
Pro	2.3	2.8
Gly	9.9	9.9
Ala	8.0	14.0
Val	1.9	6.9
Met	4.4	2.8
Ile	12.2	3.5
Leu	25.7	25.0
Tyr	5.7	5.0
Phe	2.1	7.3
Trp	2.4	2.1
His	6.9	5.8
Lys	11.7	8.7
Arg	3.1	9.5
Subunit MW (SDS gel electrophoresis)	17,000*	18,500

The analysis of *Azotobacter* bacterioferritin-cytochrome was carried out on protein samples without Fe removal. Similar analyses to those shown here on this protein by D. Jacobs and the late W. A. Bulen (D. Jacobs, personal communication) give values which differ from those reported here by less than a single residue for each amino acid. Data for horse spleen ferritin are from ref. 6.

*Obtained by the procedure of Weber, Pringle and Osborn³⁰ using, as calibrants, aldolase, pepsin, chymotrypsin, trypsin, ferritin, myoglobin, haemoglobin, lysozyme and cytochrome c.

anaerobic Biogel P-2 column such that >90% of the (ferrous) iron is maintained in the reduced protein, which elutes with the solvent front. Separate experiments demonstrate that the column easily separates reduced ferritin-cytochrome from added ferrous ions. The eluted fully reduced ferritin-cytochrome can then be rapidly reoxidised by air to a species which is spectroscopically and analytically indistinguishable from the original oxidised material, with the exception of a small loss of Fe. The *Azotobacter* protein seems to hold its iron longer in the reduced state than does mammalian ferritin.

We conclude that *A. vinelandii* produces a protein with dual bacterioferritin-cytochrome character. If the molecule resembles ferritin in quaternary structure (as seems likely from the electron microscopy), then its 24 subunits, ~12 haems and average of 1,600 Fe atoms in the oxide-hydroxide-phosphate core give a material of MW 660,000 capable of taking up or delivering up to 1,600 electrons. It differs from mammalian ferritin in that it contains haem, has a lower redox potential and holds its iron more tenaciously in the Fe^{2+} state. We can only speculate on the physiological function and biological ubiquity of this bacterioferritin-cytochrome.

The high iron content makes this protein a prime candidate for an iron storage protein of *A. vinelandii*, much as ferritins have this function in mammalian, plant and fungal systems¹⁻³. If Fe storage is indeed its physiological role, then, as has been postulated in mammalian systems¹⁸⁻²¹, iron mobilisation may be effected by the reduction of Fe^{3+} to the more labile and more soluble Fe^{2+} state. Perhaps the presence of the haem in the same molecule facilitates the reduction of the internal ferritin Fe. Why, then, is such a low potential required to mobilise the Fe? An intriguing hypothesis is that the Fe in the ferritin-cytochrome is a specific iron-storage depot for nitrogenase and its low redox potential ensures that only when the local redox potential in the cell approaches the negative values required for nitrogenase turnover (~-430 mV)²² will the iron be mobilised from the ferritin-cytochrome.

An alternative or possibly additional role of the bacterioferritin-cytochrome involves its functioning as an electron storage

protein. The ability of this single molecule to take up hundreds of electrons at a low redox potential suggests that in *Azotobacter* this protein may function to supply low potential redox equivalents for use in respiration, biosynthesis or nitrogen fixation.

Many bacteria²³, including *Azotobacter*²⁴, excrete siderophores in conditions of Fe deficiency. It seems likely, therefore, that in conditions of iron sufficiency, species other than *A. vinelandii* will also have developed the ability to make bacterioferritin. Furthermore, some of these bacterioferritins could contain haem as does the *Azotobacter* protein. Spectroscopically, the haem absorptions found in the *A. vinelandii* protein are extremely similar to those found in cytochromes *b*₁ (often of unknown physiological function) from various bacterial sources²⁵. Although none of the other cytochromes *b*₁ have been reported as containing non-haem iron, these do share with the *A. vinelandii* ferritin-cytochrome a relatively low redox potential and a tendency to be present in high MW aggregates. As cytochrome *b*₁ purification procedures often involve the use of detergents (which would cause loss of Fe³⁺ core and leave apoferritin), the possibility remains that these are the bacterioferritin-cytochrome analogues of apoferritin.

In mammalian Fe metabolism, a close relationship between ferritin and haem metabolism has been postulated in mitochondrial particles, and evidence for direct involvement of ferritin in haem biosynthesis has been considered²⁶⁻²⁹. Further studies of bacterioferritins and/or bacterioferritin-cytochromes may help to establish evolutionary links which could improve our comprehension of both microbial and mammalian iron metabolism.

We thank Deloria Jacobs and Sam Lough for assistance in protein preparation and manipulation, Dr Harry E. Calvert for the electron microscopy, Dr Kenneth F. Miller for the magnetic susceptibility studies and Drs George Lang and Kevos Spartalian of The Pennsylvania State University for informing us of the Mössbauer spectroscopy results. The amino acid analysis of *Azotobacter* bacterioferritin-cytochrome was carried out by AAA Laboratory, Mercer Island. The whole experiment is contribution 647 from the Charles F. Kettering Research Laboratory.

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Structure of the iron-sulphur clusters in *Azotobacter* ferredoxin at 4.0 Å resolution

A FERREDOXIN-LIKE protein from *Azotobacter vinelandii* having 6-7 Fe and 6-7 S²⁻ per mol and a molecular weight of 14,000 has been described by Shethna¹ and Yoch *et al.*². Its biochemical properties³, electron paramagnetic resonance and redox behaviour^{4,5}, and primary sequence⁶ have also been studied. This work has established that there are two Fe-S centres separated by 0.744 V in reduction potential, one behaving like the [Fe₄S₄S₄²⁺] cluster in high-potential iron proteins, the other displaying novel characteristics. The distribution of cysteines in the N-terminal sequence is distinctly non-homologous with clostridial ferredoxins. Extrusion of the Fe-S cores by thiol displacement produces unique species, suggesting the presence of a new chromophore structure⁷. I report here crystallographic studies of the protein in a tetragonal crystal form which have led to an electron density map at 4.0 Å resolution. This map reveals two Fe-S clusters of clearly different size and shape. It has not been previously shown that this Fe-S protein, or any other, actually contains clusters of differing structure.

The protein was isolated by the method of Shethna¹ (*A*₂₈₀/*A*₄₀₀ = 1.7-1.8) and crystallised as square bipyramidal prisms⁸. The crystals are tetragonal, space group P4₃2₁2 with *a* = 55.24 Å, *c* = 95.05 Å, and one molecule per asymmetric unit. Diffractometer data have been collected to 4.0 Å resolution from single crystals of the native and an isomorphous derivative prepared with K₂PtCl₆. Data were collected at 10 °C using CuKα radiation and an ω step-scan procedure. Bijvoet pairs were measured at ±2θ in blocks of 10 reflections. After 6 d of irradiation, decay at 4.0 Å was 20%.

Table 1 Single isomorphous replacement phase sets

Phase Set	Space group	Pt			<i>E</i>	<i>R</i> _c	⟨ <i>m</i> ⟩
		<i>x</i>	<i>y</i>	<i>z</i>			
1	P4 ₁ 2 ₁ 2	0.48	0.13	0.19	33.3	0.478	0.49
2	P4 ₁ 2 ₁ 2	-0.48	-0.13	-0.19	38.9	0.552	0.47
3	P4 ₃ 2 ₁ 2	0.48	0.13	0.19	39.4	0.547	0.47
4	P4 ₃ 2 ₁ 2	-0.48	-0.13	-0.19	33.1	0.501	0.49

$$E = \frac{\sum_n ||F_{PH}| - |F_P + f_H||}{n}$$

where *F*_P, *F*_{PH} are observed structure amplitudes for native and derivative, respectively, and *f*_H is calculated heavy atom structure amplitude, and *n* = 1,416 reflections.

$$R_c = \frac{\sum_{hkl} ||F_{PH}| - |F_P + f_H||}{\sum_{hkl} ||F_{PH}| - |F_P||}$$

for 475 centric reflections (0*kl*, *hk*0, *hhl*).

⟨*m*⟩, Mean figure of merit.

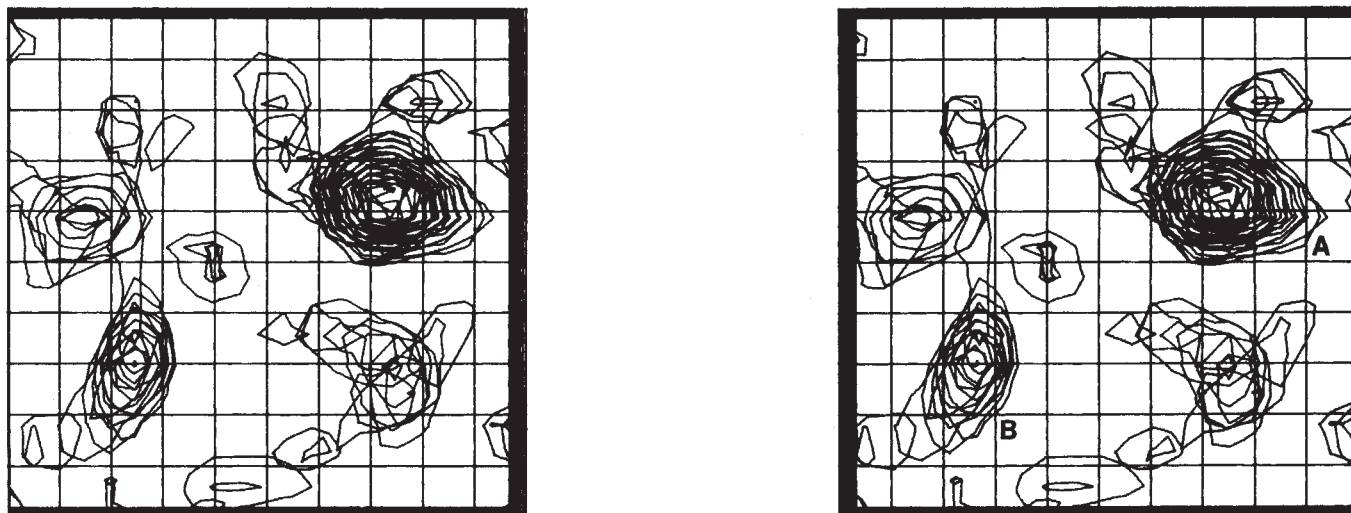


Fig. 1 Electron density map of *Azotobacter* ferredoxin. Fe-S clusters are marked A, B. Seven sections on x , 0.23–0.35; y axis vertical, 0.18–0.52; z axis horizontal, 0.23–0.43. Grid spacing ~ 1.9 Å. Contours start at 18% of highest density (cluster A) with intervals of 10%.

The isomorphous difference Patterson map for the derivative showed six peaks (average peak to background ratio 3:1) consistent with a single major Pt site. Patterson maps using data from three additional Pt-soaked crystals in trial experiments were similar. The correct hand of both the heavy atom position and the space group was determined unambiguously by using the anomalous scattering from iron. Four protein phase sets were derived using the Pt data without anomalous dispersion (Table 1). In each case Bijvoet difference⁹ and 'best' Fourier maps were computed using the native data. Phase set 1 yielded two large positive peaks in the 'best' Fourier at the Fe-S cluster sites, but large negative peaks at these sites in the Bijvoet difference map. Phase sets 2 and 3 produced uninterpretable maps. With phase set 4 both Fourier maps contained large positive peaks at the Fe-S sites at positions related by $1/2 - z$ from the set 1 maps, establishing this as the correct enantiomer. Subsequently, the heavy atom parameters and protein phases were refined in $P4_32_12$ with Pt anomalous data included (Table 2). Difference Fourier maps revealed no secondary Pt binding sites. A Bijvoet difference Fourier with Pt anomalous differences again showed positive peaks at the Fe-S sites as well as a peak at the Pt site, the highest in the map and more than twice as high as the Fe-S peaks.

The electron density map in the region of the Fe-S clusters is shown in Fig. 1. The clusters are separated by 11 Å and occur at 0.26, 0.39, 0.38 (A) and 0.30, 0.28, 0.28 (B). Several stretches of the polypeptide chain can be traced in the native map, including a right-handed helical segment. A clear solvent boundary delineates a molecule $30 \times 25 \times 35$ Å in size. The map is featureless at the Pt site, which occurs on the molecular surface 19 Å and 13 Å from clusters A and B, respectively.

coccus aerogenes ferredoxin¹⁰ and high-potential iron protein¹¹. Cluster B, however, is significantly flattened and oblate in shape, being at most 4.5 Å thick. The maximum density at its centre is 75% that of cluster A. At 5.0 Å resolution, this cluster has a similar appearance but is only 55% the height of cluster A. Density maps calculated with centric or acentric phases only also give the same image of the Fe-S clusters. Thus, the electron density cannot be interpreted in terms of a second Fe_4S_4 cube. Although it is not possible to distinguish unequivocally between other possible structures at this resolution, the density for the smaller cluster does suggest a two-iron centre, in analogy with model compounds of the type $Fe_2S_2(RS)_4$ (ref. 12). This interpretation is supported by the fact that the protein chains attach to cluster A from several directions (two shown in Fig. 1), whereas cluster B is attached only at opposite ends.

A 4Fe-2Fe model agrees with the original Fe and S^{2-} assay and is consistent with the sequence. As the crystals diffract to 2.0 Å, the resolution of this study is being extended to resolve the Fe atoms and to trace the entire polypeptide chain.

This work is supported by a grant from the NIH (GM-25672). Diffractometer and computer programs were supplied by Drs R. McClure, J. Abola and M. K. Wood.

Note added in proof: W. V. Sweeney has recently determined by flame emission spectroscopy and colorimetric analysis using 1,10-phenanthroline that *A. vinelandii* ferredoxin I contains six atoms of iron per molecule, based on an extinction coefficient at 400nm of $27 \text{ mM}^{-1} \text{ cm}^{-1}$.

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Table 2 Refinement parameters for K_2PtCl_4 derivative to 4.0 Å

A^*	62.88	f_H	126.8
x	0.4816	E	45.7
y	0.1352	R_c	0.478
z	0.3155	$\langle m \rangle$	0.66
B	28.00 Å^2	n	1416

* Occupancy on scale of f_H , E (see Table 1 for definitions).

The most striking feature of the electron density is the differing shape and weight of the Fe-S peaks. Cluster A is essentially a 6 Å diameter sphere, consistent with the expected presence of a $[Fe_4S_4S_4^{Cys}]$ cluster(s), like those seen in *Pepto-*

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reviews

Solar energy boom

D. O. Hall

Solar Energy: The Awakening Science. By D. Berham. Pp. 379. (Routledge and Kegan Paul: London, 1979.) £8.95.

WITH worldwide government-sponsored research and development on solar energy in 1979 approaching $\$1 \times 10^6$, it would be very worthwhile for anyone interested in solar energy, however remotely, to read this book. It provides an excellent and well written background to the technologies and personalities of those who have developed and are currently participating in the solar energy boom around the world.

As the United States has the largest and longest running government solar energy budget, about half the world total, its solar energy scene naturally occupies the majority of the book. However, the French solar energy scene is given wide coverage, as they have the third largest programme in the world and have had a sustained effort for many years. Unfortunately the very large Brazilian bio-fuels programme ($\$300 \times 10^6$ per year) is not dwelt on; nor is the large Saudi Arabian programme ($\$25 \times 10^6$ per year) nor the other Arab solar energy programmes which are also large. The German solar energy effort is also not discussed, even though their R & D budget is over $\$30 \times 10^6$ per year; and like the French they have substantial government tax incentives for industries and individuals to install solar energy devices. The United Kingdom solar energy effort (government R & D spending $\$3 \times 10^6$ per year and no tax incentives) commands a whole chapter. We are credited with having "the thread of originality" (a passively heated school near Liverpool, built in 1961) but "certainly do not believe in trying to solve the problem of solar energy by throwing money at it"—does this sound familiar?

I can thoroughly recommend this book. I read it from cover to cover in a short time but I am probably too interested in solar energy so as to be termed "unusual" or other less kindly epithets. The background work and interviewing which the author (a science writer for UNESCO in Paris) obviously put into the writing of the book comes across clearly. The technical details are kept to a minimum,

and the explanations of the various solar energy systems now operating, or on the drawing board, for houses, factories, swimming pools, satellites, power towers, farms, and so on, are remarkably lucid. It is therefore good for all sections of the community who want to find out what is behind all the jargon which inevitably seems to accompany a technical field. Sometimes one wished for less snippets about the motels and meals and even more about some of the personalities, but the personal flavour of writing is attractive—don't be put off by the first two or three pages (unless of course you like Brittany!) A truly excellent index provides a ready means of finding any topic, person, place, laboratory, company, committee, country (and so on) mentioned in the book. In fact just reading through the index gives one quite an overview of the varied interests and technologies in the field of solar energy.

The final chapter was completed in 1978 and gives an up-to-date view of the status of the implementation of

solar energy programmes in the USA—5,000 solar heated and 50,000 solar hot water houses ($\$30 \times 10^6$ annual market) with a projection of 2.5×10^6 solar heated houses by 1978 ($\$1 \times 10^9$ annual market). The State of California could not wait for the federal government (which at the end of 1978 offered tax credits up to \$2,200 for each solar energy installation) and became the first State to offer generous incentives—"home owners get a tax credit of 55% up to \$3,000 on a purchase and installation of a solar energy system. Businesses are also given a 25% tax credit if their systems cost more than \$6,000".

The author is not a starry-eyed "solar nut", as he asks searching questions, expresses many doubts throughout the book, and rightly stresses energy conservation techniques right from the start. □

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Medicinal botany

Healing Plants: A Modern Herbal. Edited by W. A. R. Thomson. (Macmillan: London, 1978.) £9.95.

UP to a few years ago very few books on medicinal plants had been written this century for the layman, certainly in English. There was Mrs Grieve's *A Modern Herbal*, published in 1931, as well as some charming if rather less practical books by E. S. Rohde, which came out about the same time. Mrs Grieve's work covers a wide range of plants, is packed with information, much of which is difficult to find elsewhere, and is a sensible, down-to-earth publication even if it does not contain much information on the chemical constituents of plants (a great deal of work has been done on this in the past fifty years). Nevertheless, I think that when reviewing new books on medicinal plants it is fair to use Mrs Grieve as a standard by which to judge them. The kind of people who will buy and use these new books are probably the same kind as those who bought and

used *A Modern Herbal* in the 1930s.

There seems to be a much wider interest in medicinal plants now than was common even ten years ago. This is no doubt partly a spin-off from the ecology movement but is also the result of the related desire for a simple life, especially among townspeople. Whatever the basis for this interest, it is illustrated by the increase in health food stores selling herbal remedies for a wide range of afflictions. The market for books on medicinal plants is provided with an ever-increasing flow of works of varying merit. The one reviewed here is a typical example and like the curate's egg may be described as good in parts. To start with, it is very attractive to look at; the illustrations—both colour photographs and reproductions of old botanical drawings—are on the whole excellent, as is the printing. But I found the matter contained in the book (and its layout) rather disquieting.

The book is divided into a number of alphabetical lists interspersed with chapters on healing plants and their

active principles, the heritage of folk medicine, and so on. A list is provided of plants under English common-names—not always correct—with the conditions they are held to alleviate; and under latin names with an illustration and general description that would not be very helpful to a non-botanist, and is followed by a list of complaints and illnesses, with the plants used. This list unfortunately does not always tally with the first and is followed by yet another on healing substances and their effectiveness. This last list is about the parts of the plants used, their constituents, and how they are processed. It is the most informative of the sections, but would be more helpful if sources

of both herbal and planting material were given. For example, Asiatic ginseng is listed, but where the authors' expect would-be growers to buy planting material I do not know; so far as I am aware it is only available from Japan. The book would have been far more useful if the information on each plant were all in one place, instead of being scattered throughout. Lists of suppliers—both of the dried herbs and of planting material—would have increased its value enormously. Once again Mrs Grieve wins overall.

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Plant breeding for disease resistance

Plant Breeding for Pest and Disease Resistance. By G. E. Russell. Pp. 485. (Butterworths: London, 1978.) £25.

DURING the past few years, radical changes have taken place in the strategies used by plant breeders when breeding for resistance to plant pests and diseases. Following the discovery by Biffen in 1907 that resistance to a disease was often conferred by a single gene, known as a major gene, many plant breeders used small numbers of major genes to obtain resistance. It was subsequently found, however, that resistance based on a single major gene, or a few such genes, frequently broke down within a few years of its introduction into a crop plant, due to the pathogen producing new races capable of overcoming that gene. G. E. Russell has been actively concerned in devising new breeding strategies designed to provide durable resistance, and this book reflects his experience and methods of approach.

The first part of the book is devoted to a brief but useful summary of methods used for the control of plant diseases, and to a consideration of the general principles and methods of breeding for resistance. The technical terms and concepts used in plant breeding are clearly defined: though some might not agree with all the definitions offered, they are clear and unambiguous. There is a valuable section on methods, many developed by the author and his colleagues, for the introduction of resistance into commercial crops, and for its assessment when introduced.

Parts II to V are devoted to breeding for resistance to specific groups of pathogenic agents, namely the fungi,

the bacteria, mycoplasmas and viruses, animal pests and parasitic weeds. In each part, a general introduction is followed by a detailed treatment of a few selected cases in each of which substantial progress has been made in the production of resistant cultivars of crop plants. These case histories fulfill two main functions. For the specialist student, they provide a good and usually up to date account of the present position, with adequate references to the literature. For the more general reader, they illustrate very clearly the ways in which the general principles advocated by the author can be applied to obtain durable resistance to almost any type of pest or disease, with modifications in detail rather than in principle to fit any specific case.

In the final part of the book, some general conclusions on the present position and future prospects in the field are set out. The author is hopeful that by the use of major genes, where they are proved to be durable, and by the use of more sophisticated approaches where they are not, it will be possible to control many major pests and pathogens. The casual reader may at first get the impression that it is suggested that breeding is the only satisfactory solution to pest and disease problems, but in the final section, the author is careful to emphasise that in many situations an integrated control programme involving both resistant cultivars, pesticides and biological and cultural control methods may be required.

This book is a very useful addition to the literature of plant breeding and plant pathology. It will be of value both to the student entering this field, and to the more general reader who is seeking an insight into the principles and methods of modern disease and pest resistance breeding.

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Applying digital filters

Digital Filters and their Applications. By V. Cappellini, A. G. Constantinides and P. Emiliani. Pp. 393. (Academic: London, New York and San Francisco, 1978.) £20.50; \$42.

THE appearance of this volume in a series on *Techniques in Physics* is something of a landmark, for it is only recently that filters have ceased to be studied almost exclusively by electrical engineers, and written about for readers with formal training in that field. The intensive study of two-dimensional filters is also a recent development, spurred on by the need to clean up satellite pictures and by a variety of biomedical and geological needs. In connection with these and related applications, many scientists with no engineering background have been using—and rediscovering—filters to improve their "signals", and the present authors have therefore attempted to compose a text that would appeal to such an audience as well as to engineers. This is no easy task, for the types of presentation likely to satisfy these two categories of reader are rather different, particularly in the handling of mathematical details and in the need to put the material under discussion in context. The solution adopted by the authors, who present a great deal of material with very little comment or explanatory text to help the reader to assimilate it, is not at all satisfactory. It is certainly possible to look up any number of facts about filters, both FIR and IIR, their software and hardware implementations and noise, but it is extremely difficult to get any feel for their power or suitability. The section entitled "Description of some windows" is an example of this: two formulae tell us how to distinguish a Hamming window from a Hanning window but not why two nearly identical windows should coexist. The formulae for numerous other windows are also given, sometimes with some indications about their origin but not always.

This is a disappointing book, therefore, except as an *aide-memoire* for finding formulae and definitions. The authors' object was certainly laudable but for the time being the pair of books on digital signal processing by Rabiner and Gold and by Oppenheim and Shafer (Prentice-Hall, 1975) remain the most useful source of information on these matters.

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Vibrational and electronic spectroscopy

Symmetry and Spectroscopy: An Introduction to Vibrational and Electronic Spectroscopy. By M. C. Harris and M. D. Bertolucci. Pp. 550. (Oxford University Press: New York, Oxford and London; 1978.) £15.

A NEW textbook dealing with vibrational and electronic spectroscopy, at third-year undergraduate level, with group theory as the unifying thread, has been needed for some time. This ambitious book goes somewhat further into spectroscopy than I would expect third-year undergraduates to go, from foundations which are not quite deep enough.

The book is ambitious because it aims to be self-contained, assuming little background knowledge (except some quantum mechanics). This has meant difficult decisions as to which theoretical results to explain, which to assume and which to ignore. The first chapter is an extremely readable account of groups and representations which deliberately ignores the theorems

of representation theory (the four rules) and simply states the key result, the formula for decomposing a representation, by *fiat*. The relationship between group theory and the Hamiltonian is not discussed: the student who wishes to know why molecular eigenstates necessarily have symmetry properties must look elsewhere, or simply accept that normal coordinates and molecular orbitals transform as irreducible representations. Without making the book longer, much of this theory could have been included at the expense of chapter two, a sketch of quantum mechanics which adds little to the book and incidentally contains an incorrect interpretation of the superposition principle.

The theory of molecular vibrations and of molecular orbitals provided here is rather elementary compared with the level of the spectroscopy which follows. A verbal definition of a normal coordinate without the equations of motion does not indicate clearly what a normal coordinate is, nor why it can sometimes be determined by symmetry. Similarly, without some idea of an effective one-electron potential, one cannot properly explain the Koopmans theorem nor insist that the molecular orbitals have symmetry properties.

Hückel theory is presented here without any suggestion that the Hamiltonian is not the total molecular Hamiltonian.

Most of the above criticism assumes the educational importance of principles in themselves. Of equal if not more importance for the specialist is the application of principles in puzzling; and it is here that the authors have succeeded brilliantly. Interwoven with the text are many examples of spectra (very well illustrated) from the literature and over 200 problems. The student who completes even a modest fraction of these problems should be able to construct symmetry coordinates and orbitals, determine the states which arise from electronic configurations, and decide which transitions are allowed. In short, he should be able to read the literature of vibronic spectroscopy. The theoretical aspects of this book could easily be supplemented by a lecture course. The strengths of the book lie precisely where formal lectures are often weak—in examples—and for this reason both teachers and students will find it valuable.

M. P. Melrose

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Fundamental research in gerontology

The Biology of Ageing. Edited by J. A. Behnke, C. E. Finch and G. B. Moment. Pp. 388. (Plenum: New York and London, 1978.) £11.93.

AGEING is the next growth industry, perhaps hardly surprising in Western Societies, as two of the three major scourges—famine and pestilence—have been controlled, and the third—war—is unacceptable, at least in theory. About 1 in 6 of our population is over 65. In the United States there are now 22 million people over 65 and early in the next century this figure is expected to increase to 50 millions. Little wonder that the young who have to support the more elderly amongst us are becoming concerned. What do they do with this rapidly increasing army of non-productive geriatrics—parasites on a society which they have helped to build?

Unfortunately, this book does not consider the social problems of ageing but it does deal with most other aspects. It is intended for a general audience but a good knowledge of modern biological theory is an essential prerequisite.

The contributors review most areas of interest in fundamental research in gerontology, particularly ageing in cells and molecules, and ageing in plants and lower animals, but there are also substantial sections on ageing in man and on the effects of hormones on ageing. The book concludes with a section on perspectives. As with any book with 25 contributors, the individual articles vary in quality. Some can be read with pleasure, others are rather heavy going, but all provide good background information and each gives a useful reference list for further reading.

Although ageing and death are the inevitable end-results for most metazoa, we still know very little about the mechanisms involved. It is perhaps the most intellectually challenging problem in biology, yet research on ageing forms only a very small part of the major research programmes. Few universities or medical schools consider ageing to be a basic biological process of sufficient importance to form the basis for organised courses.

This book provides an excellent basis for an introduction to modern research on ageing, but it may be somewhat too detailed to serve as a course book.

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John Maddox

Croom Helm wish to apologise to Mr John Maddox for the reference to him in connection with recombinant genetics in their book *Directing Technology*. The publishers (Croom Helm) and the Editors (Ron Johnston and Philip Gummatt) and the author of the chapter concerned (Dr Edward Yoxen of the Department of Liberal Studies in Science at the University of Manchester) unreservedly withdraw any suggestions of impropriety in Mr Maddox's role in public discussion of this issue.

Circle No. 07 on Reader Enquiry Card.

High performance liquid chromatography

Applications of High Performance Liquid Chromatography. By A. Pryde and M. T. Gilbert. Pp. 255. (Chapman and Hall: London, 1979.) £10.50.

THE authors of all compilations of the applications of any analytical chemical technique have to contend with two basic problems. In the first place only a very small part of the text will be of direct interest to any particular reader, and secondly in the case of any rapidly developing method such as high performance liquid chromatography (HPLC), there is a danger that much of the described methodology will be outdated even during the time required for publication.

The present authors have skilfully avoided both these pitfalls, the first by confining their main coverage to three fields of wide general interest—pharmaceutical analysis, biochemical analysis and methods for monitoring environmental pollution. Some other miscellaneous topics are also treated briefly. The second problem—of current relevance—has been met by providing a very complete review of the literature up to Spring, 1977. Publication delays being what they are at present, this must be considered very good.

A very short but good summary of the relevant theory, a similarly brief account of the currently available instrumentation, and a more extended and valuable section on the practice and principal modes of operation of HPLC lead into the major sections of the book.

Within the selected topics the coverage provided is excellent. For example, the 48 pages devoted to pharmaceutical analysis includes most of the classes of drugs now in general use, from antibiotics to diuretics (including a section on analysis for drugs of abuse). In many cases examples of methods of HPLC analysis for the drug metabolites are included.

The major 61-page section on applications of HPLC to biochemical analysis is particularly thorough, and includes sub-sections on lipids (including steroids and prostaglandins), polycarboxylic acids of metabolic importance, carbohydrates, biogenic amines, amino acids and proteins, nucleic acid derivatives porphyrins, and the common vitamins. It is very encouraging to find some space devoted to HPLC of proteins, as in my view the HPLC of biological macromolecules has lagged behind the much easier HPLC of small

molecules, many of which can readily be fractionated by easier (and much cheaper) methods. The intrinsically high resolution of HPLC methods should be particularly valuable for the separation of large nucleic acid and protein fragments (or even subunits) which are required for the sequencing of the intact molecules.

The brief sections on applications of HPLC to environmental monitoring and various miscellaneous separations, including *s*-isomers, provide adequate coverage for their purpose.

The book concludes with a series of appendices listing in considerable detail various types of packings for HPLC columns, and finally with a very use-

ful specific index of compounds mentioned in the text, which greatly enhances its value as a reference book. The scope of this very useful book is illustrated by the 876 quoted references, mostly subsequent to 1970. The production of the book is excellent at its price, and there are very few misprints, although I found the use of the word "colourimetric" throughout the book mildly irritating.

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Molecular photoelectron spectroscopy

Photoelectron Spectroscopy and Molecular Orbital Theory. By R. E. Ballard. Pp.192. (Adam Hilger: Bristol, 1978.) £18.

THE author of this book has appreciated the value of molecular photoelectron spectroscopy as a teaching aid in chemistry. Other books on the subject have tended to emphasise its contribution to the advancement of knowledge and have been directed primarily at researchers. He recalls the late Professor C. A. Coulson's remark about the impact of photoelectron spectroscopy: "The single result of most significance is the complete vindication of the molecular orbital description of a molecule". Molecular orbital theory is taught throughout university chemistry schools, sometimes with the clarity which the late Professor Coulson used to demonstrate, all too often, however, as a beautiful mathematical theory; that in some ways is good for the undergraduates to master, but which many find sterile because of an over-emphasis on exactitude. The aim of the present book clearly is to demonstrate that photoelectron spectroscopy and especially helium I photoelectron spectra of vapours have an important part to play in introducing undergraduates rather painlessly to some of the essential concepts of molecular orbital theory.

In order presumably not to overburden the student, a number of topics which a specialist would think essential have been omitted; notably no discussion is given of the interpretation of spectra based upon ionic states, to which reference must always ultimately be made. The author is content to emphasise only the relationship between occupied orbitals and the

bands in the photoelectron spectra. This is attempted by assembling the spectra for a rather limited range of simple molecules falling into groups of increasing complexity and then discussing these in some detail. These comparative chapters are preceded by a more general introduction to the necessary molecular orbital concepts. Some use has been made in the comparative chapters of the computer-generated drawings of Jorgensen and Salem (*The Organic Chemists' Book of Orbitals*, Academic: New York, 1973) to provide an insight into the geometrical factors which are often implicit in the details of the photoelectron spectra. This is often a most revealing juxtaposition and the reviewer feels that even more use could have been made of this to advantage. The detailed comparisons are quite restricted in their coverage, ranging from diatomics, through triatomics, molecules related to ethylene, and some tetrahedral and octahedral molecules. Within these groupings are, however, to be found a number of examples of substances whose electronic structure will have been a puzzle at one time or another to most chemistry undergraduates.

The importance of the finer detail in the spectra is not treated in depth. The section on vibrational fine structure is perhaps less well illustrated in certain respects than other areas, both with regard to selection of examples and more particularly the quality of reproduction of some of the spectra. In this area in particular spectral resolution and the fineness of detail which can be read into many spectra are of fundamental importance; their proper discussion requires both well reproduced examples and a proper treatment of the concept of the ionic state manifold, which, as remarked above, is a significant omission.

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